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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 C.F.R. § 1.53(c).

Docket Number	00742/074001			Type a plus sign (+) inside this box -->	+
INVENTOR(S)/APPLICANT(S)					
LAST NAME	FIRST NAME	MIDDLE INITIAL	RESIDENCE (CITY AND EITHER STATE OR FOREIGN COUNTRY)		
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TITLE OF THE INVENTION (280 characters max)					
INHIBITORS OF THE MAP KINASE PATHWAY					
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STATE	MA	ZIP CODE	02110-2214	COUNTRY	USA
ENCLOSED APPLICATION PARTS (check all that apply)					
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<input checked="" type="checkbox"/> A check or money order is enclosed to cover the filing fees.			FILING FEE AMOUNT	\$80.00	
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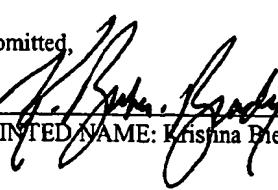
The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

No.

Yes, the name of the U.S. Government agency and the Government contract number are: **NIH CA-46595**

Applicant claims small entity status under 37 C.F.R. § 1.27.

Respectfully submitted,

SIGNATURE: 
TYPED OR PRINTED NAME: Kristina Bieker-Brady, Ph.D.

DATE: July 7, 2003
REGISTRATION NO.: 39,109



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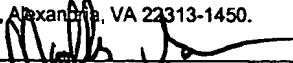
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PROVISIONAL APPLICATION

UNDER 37 C.F.R. § 1.53(c)

APPLICANT : John Blenis and Leon O. Murphy

TITLE : INHIBITORS OF THE MAP KINASE PATHWAY

INHIBITORS OF THE MAP KINASE PATHWAY

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This invention was funded by grant CA-46595 from the National Institute of Health. The government may have certain rights in the invention.

Field of the Invention

10 This invention relates to the development and use of human therapeutics that inhibit intracellular signaling via the MAP kinase pathways.

Background of the Invention

15 The evolutionarily conserved Ras-MAPK signaling network regulates diverse biological processes such as cell proliferation, differentiation, migration, and survival. Many of the regulators and effectors within this network have been implicated in diverse pathological processes. MAP kinases and their targets have been identified as, for example, potent oncogenes or tumor suppressor genes and proinflammatory mediators.

20 Normally, the MAPK network is activated when growth factors or hormones bind to cell surface receptors. The extracellular signal is amplified and converted into an appropriate biological response. However, dysfunction of any component of the signaling pathway may result in a pathological condition. Cancer, for example, is a disease marked by the uncontrolled growth of abnormal cells. Cancerous cells have overcome the barriers imposed in normal cells, which have a finite lifespan, and grow indefinitely. As 25 the growth of cancer cells continues, genetic alterations can accrue and persist so that the cancerous cell displays increasingly aggressive growth phenotypes. If left untreated, metastasis, the spread of cancer cells to distant areas of the body by way of the lymph system or bloodstream, may ensue, destroying healthy tissue and, ultimately, leading to

death. According to a recent American Cancer Society study, at least 1,268,000 new cancer cases are expected to be diagnosed in the United States in any given year.

However in cancer cells, mutations in upstream activators of MAPK, such as Ras or Raf, lead to constitutive signaling even in the absence of growth factors.

5 Constitutively activating mutations in Ras are detected in at least 30% of all human malignancies but are present in especially high levels in colon (50%) and pancreatic cancers (90%). The activation kinetics of the ERK1/2-MAPK signaling pathway have also been associated with distinct biological outcomes. In fibroblasts, sustained ERK1/2 activation over several hours induces entry into S phase of the cell cycle while transient 10 (20-30 min) activation does not.

In various cell types, the ERK1/2 pathway also has a critical role in regulation of nucleotide biosynthesis, transcription, migration, cell survival, differentiation and adaptive responses. Specifically, ERK signaling can control cardiomyocyte cell growth and the response to ventricular heart failure, cell survival in atherosclerosis, various 15 metabolic processes such as glucose uptake, protein synthesis and leptin signaling, regulation of the immune response such as in T cell activation and inflammatory cytokine signaling, and mediating the effect of neurotransmitters that control memory and behavior. ERK signaling also can control the induction of genes that are required for establishing circadian rhythms.

20 Accordingly, small molecule drugs that can selectively inhibit regulatory proteins within the ERK1/2-MAPK pathway have enormous therapeutic potential. General MAPK inhibitors, however, are likely to be toxic due to the many metabolic and proliferative functions regulated by this pathway in healthy cells. ERK1/2 specifically recognizes some physiological substrates through the presence of ERK1/2 docking sites 25 in substrates (Jacobs *et al.*, 1999; Tanoue *et al.*, 2000). At least two classes of docking site have been identified and are known as the D-box and DEF domain.

Substrate docking directs ERK1/2 to phosphorylate specific amino acids known to regulate the biological function of the substrate. Interaction of ERK1/2 with the D-box

docking site is required for ERK's initial activation by MEK, as well as its inactivation by phosphatases (Tanoue *et al.*, 2000). By contrast, the DEF domain appears to be mainly found in downstream targets of ERK1/2 (Jacobs *et al.*, 1999).

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Summary of the Invention

We have discovered that MAP kinases (e.g., extracellular signal-regulated kinase 1/2 (ERK1/2)), bind to certain target proteins (e.g., immediate early gene (IEG) products) through a DEF domain, causing the phosphorylation of target residues and a resulting biological effect (e.g., progression through the cell cycle). Thus, a variety of human 10 diseases may be treated by blocking the interaction of MAP kinases with the DEF domain of target proteins.

Accordingly, in one aspect, the present invention provides for a method of identifying therapeutic compounds that affect the MAP kinase-DEF domain interaction. The method consists of the steps of: (i) providing test cells that express a target protein 15 having a DEF domain and capable of promoting progression through the cell cycle; (ii) culturing the test cells in the presence of a growth factor, cytokine, tumor promoter, or oncogene under conditions that activate a target kinase; (iii) contacting the test cells with the candidate compound; (iv) assessing the binding of the MAP kinase to the target protein, wherein a candidate compound that inhibits the binding is a therapeutic 20 compound. Desirably, the test cells are mammalian; most desirably human. Suitable test cells include, for example, a primary cell line, an immortalized cell line, or a tumor cell line. Fibroblasts are particularly useful test cells, but any mammalian cell type can be used because IEGs are ubiquitously expressed. Useful growth factors, cytokines, tumor promoters, and oncogenes include, for example, epidermal growth factor (EGF) and EGF- 25 related factors including, for example, transforming growth factor α (TGF α), heparin-binding-like EGF, heregulin, amphiregulin, epiregulin, cripto, platelet derived growth factor (PDGF), including PGDF-AA, PGDF-BB, and PGDF -CC, insulin, insulin-like growth factors (IGFs), fibroblast growth factors (FGFs), colony stimulating factor (CSF),

and heaptocyte growth factor (HGF). Useful cytokines include, for example, the chemokines, interleukins, and lysophosphatidic acid (LPA). Useful tumor promoters include, for example, phorbol esters, phosphatase inhibitors such as okadaic acid, microcystin, vanadate, hydrogen peroxide, and calyculin A. Useful oncogenes include, 5 for example, Erb2/neu, sis, kit, Ras, Raf, PI3-kinase, and PTEN. Epidermal growth factor (EGF) and platelet-derived growth factor (PDGF) are particularly useful growth factors. In other embodiments in which the target protein is c-Fos, the binding of the MAP kinase to c-Fos is assessed by measuring the phosphorylation of T325 or T331. Preferably, this is performed using a phospho-T325-specific antibody.

10 In another aspect, the invention provides a method for identifying a therapeutic compound by (i) providing a polypeptide that contains a DEF domain, a MAP kinase, and a candidate compound, (ii) mixing the polypeptide, the MAP kinase, and the candidate compound, (iii) measuring the binding of the MAP kinase to the candidate compound, and (iv) identifying the candidate compound as a therapeutic compound, wherein the 15 candidate compound inhibits the binding measured in step (iii), compared to the binding of the MAP kinase to the polypeptide in the absence of the candidate compound. In desirable embodiments, the target protein further contains a fluorescent moiety (e.g., fluorescein).

In preferred embodiments of the previous two aspects, the MAP kinase is ERK1/2. 20 In other desirable embodiments, the target proteins are members of the Fos, Jun, and Myc family proteins. Specifically, desirable target proteins include c-Fos, Fra-1, Fra-2, cMyc, N-Myc, JunD, JunB, c-Jun, in addition to Egr-1 and mPer1. In one embodiment, the target protein contains the sequence of a protein identified in Table 1 or 2 and the identified therapeutic is useful for treating cancer. In another embodiment, the target 25 protein contains the sequence of a protein identified in Table 3 and the identified therapeutic is useful for treating a cardiovascular disease. In another embodiment, the target protein contains the sequence of a protein identified in Table 4 and the identified therapeutic is useful for treating an inflammatory disorder. In another embodiment, the

target protein contains the sequence of a protein identified in Table 5 and the identified therapeutic is useful for treating a metabolic disorder. In another embodiment, the target protein contains the sequence of a protein identified in Table 6 and the identified therapeutic is useful for treating a neuropathy or a behavioral disorder. In another 5 embodiment, the target protein contains the sequence of a protein identified in Table 7 and the identified therapeutic is useful for treating a sleep disorder. In other embodiments, the target protein contains a DEF domain having the amino acid sequence F/Y-X-F/Y-X (SEQ ID NO: 28), preferably the sequence is F/Y-X-F/Y-P (SEQ ID NO: 29), more preferably the sequence is F-X-F-P (SEQ ID NO: 1). Assessment of target 10 residue phosphorylation is desirably performed using a phospho-specific antibody.

In another aspect, the invention provides a method for treating cancer in a mammal (e.g., human) by administering a therapeutically effective amount of a compound that inhibits the binding of a MAP kinase to the DEF domain of a target protein. Preferably, the MAP kinase is ERK 1/2, and the target protein is a member of the Fos, Jun, and Myc 15 family proteins including, for example, c-Fos, Fra-1, Fra-2, cMyc, N-Myc, JunD, JunB, and c-Jun. Alternatively, the target protein is one identified in Tables 1 or 2. Alternatively, the compound inhibits the binding of a MAP kinase to a DEF domain having the amino acid sequence F/Y-X-F/Y-X (SEQ ID NO: 28), preferably the sequence is F/Y-X-F/Y-P (SEQ ID NO: 29), more preferably the sequence is F-X-F-P (SEQ ID 20 NO: 1).

In another aspect, the invention provides a method for treating a cardiovascular disease in a mammal (e.g., human) by administering a therapeutically effective amount of a compound that inhibits the binding of a MAP kinase to the DEF domain of a target protein. Preferably, the MAP kinase is ERK 1/2, and the target protein is one identified 25 in Table 3. Alternatively, the compound inhibits the binding of a MAP kinase to a DEF domain having the amino acid sequence F/Y-X-F/Y-X (SEQ ID NO: 28), preferably the sequence is F/Y-X-F/Y-P (SEQ ID NO: 29), more preferably the sequence is F-X-F-P (SEQ ID NO: 1).

In another aspect, the invention provides a method for treating an inflammatory disorder in a mammal (e.g., human) by administering a therapeutically effective amount of a compound that inhibits the binding of a MAP kinase to the DEF domain of a target protein. Preferably, the MAP kinase is ERK 1/2, and the target protein is one identified in Table 4. Alternatively, the compound inhibits the binding of a MAP kinase to a DEF domain having the amino acid sequence F/Y-X-F/Y-X (SEQ ID NO: 28), preferably the sequence is F/Y-X-F/Y-P (SEQ ID NO: 29), more preferably the sequence is F-X-F-P (SEQ ID NO: 1).

In another aspect, the invention provides a method for treating a metabolic disorder in a mammal (e.g., human) by administering a therapeutically effective amount of a compound that inhibits the binding of a MAP kinase to the DEF domain of a target protein. Preferably, the MAP kinase is ERK 1/2, and the target protein is one identified in Table 5. Alternatively, the compound inhibits the binding of a MAP kinase to a DEF domain having the amino acid sequence F/Y-X-F/Y-X (SEQ ID NO: 28), preferably the sequence is F/Y-X-F/Y-P (SEQ ID NO: 29), more preferably the sequence is F-X-F-P (SEQ ID NO: 1).

In another aspect, the invention provides a method for treating a neuropathy or a behavioral disorder in a mammal (e.g., human) by administering a therapeutically effective amount of a compound that inhibits the binding of a MAP kinase to the DEF domain of a target protein. Preferably, the MAP kinase is ERK 1/2, and the target protein is one identified in Table 6. Alternatively, the compound inhibits the binding of a MAP kinase to a DEF domain having the amino acid sequence F/Y-X-F/Y-X (SEQ ID NO: 28), preferably the sequence is F/Y-X-F/Y-P (SEQ ID NO: 29), more preferably the sequence is F-X-F-P (SEQ ID NO: 1).

In another aspect, the invention provides a method for treating a sleep disorder in a mammal (e.g., human) by administering a therapeutically effective amount of a compound that inhibits the binding of a MAP kinase to the DEF domain of a target protein. Preferably, the MAP kinase is ERK 1/2, and the target protein is one identified

in Table 7. Alternatively, the compound inhibits the binding of a MAP kinase to a DEF domain having the amino acid sequence F/Y-X-F/Y-X (SEQ ID NO: 28), preferably the sequence is F/Y-X-F/Y-P (SEQ ID NO: 29), more preferably the sequence is F-X-F-P (SEQ ID NO: 1).

5 Particularly useful DEF domain inhibitors for any of the therapeutic methods are polypeptides having the sequence F/Y—X—F/Y—X (SEQ ID NO: 28; “naked DEF domains”) and chimeric proteins that contain a DEF domain inserted into a non-target protein. In preferred embodiments, the DEF domain has the sequence F/Y-X-F/Y-P (SEQ ID NO: 29), more preferably the sequence is F-X-F-P (SEQ ID NO: 1). The most 10 desirable chimeric proteins are based on non-target proteins that affect the pharmacokinetic or pharmacodynamic properties compared to administering the naked DEF domain alone. For example, DEF domains may be incorporated into serum albumin or cereoplasmin.

15 The compound is administered in an amount, frequency, and duration that is therapeutically effective for treating the diagnosed condition. Desirably, the compound is administered in an amount between 0.01 and 3000 mg/day, more preferably, in an amount between 0.1 and 2000 mg/day, either orally or by injection (i.e., intravenous, intramuscular, or subcutaneous). Alternatively, the compound can be administered as a 0.5% to 25% topical formulation.

20 Therapy may be provided in any appropriate location: at home, the doctor’s office, a clinic, a hospital’s outpatient department, or a hospital. Treatment generally begins at a hospital so that the doctor can observe the therapy’s effects closely and make any adjustments that are needed. The duration of the therapy depends on the condition being treated, the age and condition of the patient, the stage and type of the patient’s disease, 25 and how the patient’s body responds to the treatment. Drug administration may be performed at different intervals (e.g., daily, weekly, or monthly).

 In another aspect, the invention provides an antibody that specifically binds to phospho-T-325 c-Fos. The antibody may be monoclonal or polyclonal.

In another aspect, the invention provides a pharmaceutical formulation that contains a therapeutic compound identified by either of the first two aspects of the invention, and a pharmaceutically acceptable carrier. The pharmaceutical formulation may be suitable for oral administration, injection, or topical application.

5 By "specifically binds to phospho-T-325 c-Fos," when referring to the antibodies of this invention, is meant an antibody that binds with high affinity ($<10^{-8}$ M) to native c-Fos in which the threonine at amino acid position 325 is phosphorylated, but does not significantly bind to c-Fos in which the T325 is unphosphorylated. Desirably, the difference in specificity of antibody binding between phospho-T-325 c-Fos and the 10 unphosphorylated form is at least 5-fold, 10-fold, 25-fold, 50-fold, 100-fold, or 1000-fold.

By "DEF domain" is meant a polypeptide having the amino acid sequence: F/Y—X₁—F/Y—X₂ (SEQ ID NO: 28), wherein F is phenylalanine, Y is tyrosine, P is proline, and X₁ and X₂ are any naturally-occurring or non-naturally-occurring amino acids. Desirably, X₂ is proline.

15 By "target protein" is meant any protein that contains a DEF domain capable of binding a target kinase (e.g., a MAP kinase). Desirable target proteins are phosphorylated by the MAP kinase ERK1/2 following ERK1/2 binding to the DEF domain. Target proteins include, for example, gene products of the immediate early genes from the Fos, Myc, and Jun families, proteins identified in Tables 1-7, or chimeric or synthetic proteins 20 into which a DEF domain has bee inserted by artifice. Specific target proteins include, for example, c-Fos, Fra-1, Fra-2, cMyc, N-Myc, JunD, JunB, c-Jun, Egr-1, and mPer1.

By "target residue(s)" is meant one or more residues of a target protein that are N-terminal to the DEF domain and that are phosphorylated as a result of the binding of a MAP kinase. Target residues include, for example, T325 and T331 of c-Fos.

25 By "primed," when referring to a target protein, is meant a phosphorylation event that makes a DEF domain available for binding of a MAP kinase. Thus, the amino acid residues that are the subject of a "priming" modification are not the same as the target

residues. For example, c-Fos is primed when S362 and/or S374 are phosphorylated or substituted for aspartate or glutamate.

By "target kinase" is meant a protein kinase that is capable of binding a DEF domain and phosphorylating a target residue. Target kinases include the MAP kinases such as ERK1/2, for example. Thus, an "activated target kinase" is one that itself has undergone a post-translational modification causing an increase in kinase activity and/or inducing a change in subcellular localization. For example, in order to be fully activated and translocated from the cytoplasm to the nucleus, ERK1/2 is phosphorylated.

By "MAP kinase" is meant a kinase that recognizes and transfers phosphate to a phosphoacceptor amino acid with the general consensus Ser/Thr-Pro.

By "DEF domain inhibitor" is meant any chemical compound (i.e., polypeptide or non-peptide) that inhibits the interaction of a target kinase (i.e., ERK1/2 or RSK) with the DEF domain of a target protein.

By "assessing," when referring to a step in a method, is meant either qualitative or quantitative assessment of an appropriate endpoint. For example, a qualitative assessment of ERK1/2 phosphorylation is visualization of the ERK localization in the nucleus following an appropriate stimulus. Quantitative assessment may be performed, for example, using Western blotting or enzyme assay.

By "cancer" is meant neoplastic cells multiplying in an abnormal manner. In a cancer, growth is uncontrolled and progressive, and occurs under conditions that would not elicit, or would halt the multiplication of non-cancerous cells. Cancer includes, for example, leukemias and lymphomas (Hodgkin's disease, non-Hodgkin's disease), as well as solid tumors such as sarcomas and carcinomas (e.g., fibrosarcoma, liposarcoma, osteogenic sarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, Wilm's tumor, cervical cancer, uterine cancer, testicular cancer, small and/or non-small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, schwannoma, meningioma,

melanoma, neuroblastoma, and retinoblastoma.

By "treating cancer" is meant a therapy that measurably slows, stops, or reverses the growth rate of the cancer (i.e., neoplastic cells) *in vivo*. Desirably, a slowing of the growth rate is by at least 20%, 30%, 50%, or even 70%, as determined using a suitable 5 assay for determination of cell growth rates (e.g., a cell growth assay described herein). Typically, a reversal of growth rate is accomplished by initiating or accelerating necrotic or apoptotic mechanisms of cell death in the neoplastic cells, resulting in a shrinkage of the neoplasm. Efficacy of a treatment may be measured by any means known to those skilled in the art including tumor imaging or measurement of neoplastic markers.

10 By "cardiovascular disease" is meant ischemic heart disease, ventricular heart failure, cardiac hypertrophy, hypertension, and atherosclerosis.

By "inflammatory disorder" is meant any condition that is characterized by inflammation as a primary or secondary symptom. Inflammatory disorders include, for example, allergic or autoimmune disorders, anaphylaxis, and septic shock. Examples of 15 allergic disorders include allergic rhinitis, asthma, atopic dermatitis, and food allergies. Examples of autoimmune disorders include, but are not limited to, type 1 insulin-dependent diabetes mellitus, inflammatory bowel disease, Crohn's disease, ulcerative colitis, dermatitis, meningitis, thrombotic thrombocytopenic purpura, Sjögren's syndrome, encephalitis, uveitis, leukocyte adhesion deficiency, rheumatoid and other forms of 20 immune arthritis, rheumatic fever, Reiter's syndrome, psoriatic arthritis, progressive systemic sclerosis, primary biliary cirrhosis, pemphigus, pemphigoid, necrotizing vasculitis, myasthenia gravis, multiple sclerosis, lupus erythematosus, polymyositis, sarcoidosis, granulomatosis, vasculitis, pernicious anemia, CNS inflammatory disorder, antigen-antibody complex mediated diseases, autoimmune hemolytic anemia, 25 Hashimoto's thyroiditis, Graves disease, habitual spontaneous abortions, Reynard's syndrome, glomerulonephritis, dermatomyositis, chronic active hepatitis, celiac disease, autoimmune complications of AIDS, atrophic gastritis, ankylosing spondylitis and Addison's disease.

By "metabolic disorder" is meant a disease that interferes with the normal metabolic function of cells, tissues or organs. Metabolic disorders include, for example, diabetes, obesity, jaundice, polycystic kidney and hepatic disease, pancreatitis, Graves' disease, and Werner's syndrome. Metabolic diseases may also arise as secondary complications of another disease such as one involving a tumor. For example, cachexia or muscle wasting, and metabolic and digestive complications often arise from the presence of pancreatic, colonic, stomach, hepatic and hepatocellular tumors.

5 By "neuropathy" is meant any condition of the central or peripheral nervous system characterized by axonal loss that may or may not be accompanied by neuronal loss. Neuropathies specifically include conditions affecting sensory and motor neurons and include, for example, diabetic neuropathy, muscular dystrophy, Williams Beuren's Syndrome.

10 By "behavioral disorder" is meant any condition affecting motivation, emotion, learning, or memory. Behavioral disorders are also meant to broadly encompass neurodegenerative diseases. Thus, behavioral disorders include, for example, psychosis, schizophrenia, autism, Down's Syndrome, Parkinson's Disease, Alzheimer's Disease, epilepsy, Cockayne syndrome, bipolar disorders, and depression. Also included are addictions including, for example, addictions to opiates and barbiturates.

15 By "sleep disorder" is meant any condition that primarily affects sleep and consciousness. Sleep disorders include, for example, advanced sleep phase syndrome, delayed sleep phase syndrome, insomnia and narcolepsy.

20 By "a therapeutically effective amount" is meant the amount of a compound required to treat cancer (i.e., inhibit the growth of the neoplastic cells). The effective amount of active compound(s) used to practice the present invention for therapeutic treatment of neoplasms (i.e., cancer) varies depending upon the manner of administration, the age, body weight, and general health of the subject. Ultimately, the attending physician or veterinarian will decide the appropriate amount and dosage regimen.

Brief Description of Drawings

FIGURE 1 is a series of photomicrographs showing the differential responsiveness of Swiss 3T3 fibroblasts to growth factors. FIGURE 1A shows quiescent Swiss 3T3 cells (-) that were treated with EGF (25 ng/ml) or PDGF (20 ng/ml) for 20 h and then 5 processed for BrdU incorporation, as described below. FIGURE 1B shows quiescent Swiss 3T3 cells that were treated with PDGF or EGF for the indicated times and ERK1/2 and RSK kinase activities were determined using immunocomplex kinase assays. The fold activation at each time is indicated above each lane. FIGURE 1C is the indirect immunofluorescence detection of hyperphosphorylated activated ERK1/2 in Swiss 3T3 10 cells treated with EGF or PDGF. FIGURE 1D is the indirect immunofluorescence detection of c-Fos in Swiss 3T3 cells treated with EGF or PDGF.

FIGURE 2A is an illustration showing the residues in c-Fos that are phosphorylated by RSK and ERK1/2 *in vivo*. FIGURE 2B shows the electrophoretic separation of cell extracts from parallel cultures of 208F fibroblasts stably expressing 15 Fos-WT (WT), Fos-AA (AA) or Fos-DD (DD) that were metabolically labelled with ³⁵S-methionine or ³²P-orthophosphate and cultured with or without 10% FBS for 15 min. Fos proteins were immunoprecipitated from cell lysates and analysed by SDS-PAGE. The serum-stimulated phosphorylation of Fos-DD was consistently two to threefold greater than Fos-AA and arrows indicate the major mobilities observed after stimulation. 20 FIGURE 2C is Western blot from NIH 3T3 cells transfected with Fos-WT (WT), Fos-AA (AA) or Fos-DD (DD) were serum-starved and then pre-treated with 5 µM UO126 (+) or 0.1% DMSO (-) for 30 min before treatment with EGF (+) for 5 min. FIGURE 2D is an autoradiogram of an *in vitro* phosphorylation of the indicated (His)₆-Fos proteins by endogenous ERK1/2 from quiescent or EGF-stimulated NIH 3T3 cells. Results shown 25 are representative of three independent experiments. Fos-EE (S362E/S374E) was used as primed c-Fos for *in vitro* phosphorylation studies.

FIGURE 3A is an illustration that details the DEF domain at the C-terminus of c-Fos. The phosphorylation of Fos-EE in the absence of peptide competitor is expressed

as 100%. *In vitro* phosphorylation of (His)₆-Fos-EE was performed as described below. The data shown are the means \pm SEM from three experiments. FIGURE 3B is a graph showing the inhibition of Fos-EE phosphorylation by peptides containing a DEF domain (FQFP; SEQ ID NO: 3) or a mutated DEF domain (AQAP; SEQ ID NO: 4). FIGURE 3C 5 is a graph showing the inhibition of Fos-EE phosphorylation by peptides containing the c-Fos DEF domain (FTYP; SEQ ID NO: 2) or a mutated DEF (ATYP; SEQ ID NO: 5). FIGURE 3D shows the results of a Western blot of NIH 3T3 cells transfected with the indicated FLAG-Fos-DD (DD) alleles were left quiescent (-) or were stimulated (+) with EGF for 5 min before lysis. Arrows indicate the major Fos-DD mobilities. FIGURE 3E 10 is a Western blot of cells transfected with the indicated FLAG-Fos alleles. Arrows show the three major Fos-WT mobilities associated with growth factor stimulation.

FIGURE 4A is an illustration identifying the ERK1/2 phosphorylation sites N-terminal to the DEF domain in c-Fos. *In vitro* phosphorylation of (His)₆-Fos-EE 15 proteins by activated (His)₆-ERK1/2 was performed. The phosphorylation of the Fos-EE point mutants is expressed as a percentage of Fos-EE (100%). The data shown are the means \pm SEM from three experiments. FIGURE 4B is a Western blot of NIH 3T3 cells that were transfected with the indicated FLAG-Fos-DD (DD) alleles. Cells were treated with EGF for 5 min or left untreated. FIGURE 4C is a Western blot of cells transfected with the indicated FLAG-Fos alleles and treated as in Figure 3B.

FIGURE 5A is an illustration identifying the phospho-Thr 325 peptide used to 20 generate the phospho-Thr-325-specific anti-c-Fos antiserum. FIGURE 5B is a Western blot of NIH 3T3 cells that were transfected with the indicated c-Fos alleles or with vector alone. Extracts were prepared from quiescent (-) or EGF-stimulated (+) cells and analyzed using either the anti-c-Fos antibody or the phospho-Thr 325 antiserum. Results 25 shown are representative of three independent experiments. FIGURE 5C is a Western blot of Δ B-Raf-ER NIH 3T3 cells transfected with Fos-WT or Fos-AA that were either starved and left untreated (0) or treated with 1 μ M tamoxifen (TAMX) for the indicated times before lysis. The *in vivo* phosphorylation of Thr 325 in Fos-WT and Fos-AA was

analyzed by western blotting using the phospho-Thr 325-specific antiserum. FIGURES 5D and 5E are Western blots demonstrating the *in vivo* mitogen-regulated phosphorylation of Thr 325 in the context of the Fos DEF domain mutants. NIH 3T3 cells expressing the indicated Fos proteins were treated as in the same manner as for FIGURE 3B, and 5 extracts analyzed for phosphorylation of Thr 325.

FIGURE 6A is a Western blot of quiescent Swiss 3T3 cells were treated with EGF (25 ng/ml) for the indicated times. Lysates were probed for endogenous c-Fos, Thr 325 phosphorylation in c-Fos. FIGURE 6B is a Western blot of Swiss 3T3 cells that were treated with PDGF-BB (20 ng/ml) and processed as in described for Figure 6A. Results 10 shown are representative of three experiments. FIGURE 6C is a Western blot of quiescent Swiss 3T3 cells that were treated with PDGF for 60 min and then treated with UO126 (5 μ M), as indicated, or with DMSO (lanes 3-8) for the remainder of the experiment. The expression and phosphorylation of endogenous c-Fos was visualized as in above. FIGURE 6D is an autoradiogram showing the kinase activities of endogenous 15 ERK1/2 and RSK in cell lysates from Figure 6C. The fold activity is provided above each lane.

FIGURE 7A is a bar graph showing the AP-1 transcriptional activity of the indicated c-Fos alleles in HeLa cells. AP-1 luciferase activity in cells expressing Fos-WT is expressed as 100%, and the data shown are from five individual experiments. FIGURE 20 7B is a photomicrograph showing the expression of endogenous c-Fos in quiescent (-) or serum-stimulated (+) pMV7-infected 208F fibroblasts (vector), assayed by immunofluorescence microscopy. To examine the expression of Fos-WT, Fos^{Y45A} or Fos^{T325A/T331A} in G418-resistant 208F fibroblasts, cells were serum-starved for 24 h before fixation and processing for immunofluorescence microscopy using the anti-Fos antibody. FIGURE 7C is a Western blot of c-Fos protein expression in quiescent 208F cells. 25 FIGURE 7D is a bar graph showing the anchorage-independent growth of G418-resistant pools of 208F cells stably expressing pMV7 (vector) or the indicated FLAG-Fos alleles. The data are expressed as a percentage of the number of colonies formed by cells

expressing Fos-WT (100%) and represent the mean \pm SEM from six experiments performed in duplicate.

5 FIGURE 8 is a schematic diagram of the molecular interpretation of ERK1/2 signal duration. Growth factor stimulation (stimulus) causes activation of signaling pathways (signals) that result in rapid transcriptional induction of immediate early genes (response). The duration of ERK1/2 signaling is then interpreted by immediate early gene products that contain DEF domains (signal sensors). ERK1/2-docking to the DEF domain results in sensor phosphorylation. Docking and phosphorylation alters its biological activity, and this dictates the biological outcome. TF, transcription factor.

10 FIGURE 9A is a photomicrograph showing the nuclear accumulation of active ERK1/2 and c-Fos in growth factor-treated Swiss 3T3 cells. These photomicrographs are enlargements of the images of Figure 1C in order to visualize the cellular distribution of activated ERK1/2 and nucleolar structures. FIGURE 9B is a photomicrograph of 15 quiescent Swiss 3T3 cells treated with PDGF for 75 minutes followed by the addition of cyclohexamide (+) or vehicle (-). Cells were processed for c-Fos immunofluorescence 90, 180 or 300 minutes after PDGF stimulation. In control experiments (bottom two panels), cells were incubated with cyclohexamide or vehicle for 20 minutes prior to treatment with PDGF for 90 minutes.

20 FIGURE 10A is a Western blot of NIH3T3 cells transiently transfected with FosWT or FosDD were left quiescent or treated with EGF (50 ng/ml, 5 min) prior to lysis. An aliquot from each cell extract was incubated in the presence or absence of λ protein phosphatase (P'ase) for 30 minutes on ice. Data shown is representative of three separate experiments. FIGURE 10B is a Western blot of NIH3T3 cells stably expressing Δ B-Raf:ER that were transfected with FosWT, FosAA or FosDD. Cells were deprived of 25 serum growth factors, pre-treated with 5 μ M UO126 (+) or 0.1% DMSO (-) for 30 minutes prior to treating with tamoxifen (TAMX, 1 μ M) for 15 minutes prior to cell lysis.

FIGURE 10C is a Western blot showing the phosphorylation of (His)₆-FosWT, AA or EE or MBP by Flag-ERK5. Active (+) and inactive ERK5 (-) was obtained by

coexpressing Flag-ERK5 and HA-MEK5(D) or control vector, respectively, in 293 cells followed by immunoprecipitation of Flag-ERK5 from cell lysates using the M2 anti-Flag monoclonal antibody. Autophosphorylation (auto-P) of ERK5 in kinase reactions is indicated. Together, these data demonstrate that the phosphorylation of primed c-Fos is 5 regulated by the Raf/Mek/ERK pathway.

FIGURE 11 is a Western blot of quiescent Rat-1 cells that were treated with the indicated concentrations of LPA for various times. The activation kinetics of ERK1/2 demonstrates that c-Fos is a sensor for sustained ERK1/2 signaling in Rat-1 fibroblasts. The data shown is representative of at least three individual experiments.

10 FIGURE 12 is a series of cell culture plates, fixed and then stained with Giemsa to visualize foci. The indicated Fos proteins were stably expressed in 208F cells and cultured for four weeks in regular culture medium. Identical data was obtained from five separate experiments. Thus, substituting aspartic acid for T235 and T331 in c-Fos promotes Fos-mediated transformation.

15 FIGURES 13A-C are a series of Western blots showing the regulation of ectopically expressed Fos family proteins (c-Fos, Fra-1, and Fra-2) by the ERK1/2 pathway in NIH 3T3 cells. Cells were treated with or without EGF (50 ng/mL) for 5 minutes prior to cell lysis. Where indicated, UO126 (5 mM) was added to cells 30 minutes before adding EGF. EGF treatment in the absence of UO126 activated ERK1/2, 20 as demonstrated by the mobility shift to a higher molecular weight. ERK1/2 activation resulted in phosphorylation of the target amino acid, T325, of c-Fos (Figure 13A). ERK1/2 activation of Fra-1 (Figure 13B) and Fra-2 (Figure 13C) is also demonstrated by the observed mobility shift.

25 FIGURE 14A is a sequence alignment of c-Fos, Fra-1, and Fra-2 (SEQ ID NO: 25-27, respectively) demonstrating a high degree of sequence identity in the C-termini. Fra-1 and Fra-2 have ERK1/2 and RSK priming phosphorylation sites in addition to DEF domains. FIGURE 14B is a Western blot of NIH 3T3 cells transfected with the indicated constructs and deprived of serum growth factors for 24 hours. These results demonstrate

that mutations in the DEF domains of Fra-1 and Fra-2 inhibit the ERK1/2-mediated mobility shift (compare to Figures 13B and 13C).

FIGURE 15A is a Western blot of c-Myc immunoprecipitation from NIH 3T3 cells transfected with pcDNA3 (vector) or c-Myc and deprived of serum growth factors. EGF and UO126 were used to treat cells as described in Figure 13. FIGURE 15B is a Western blot from cells transfected with the indicated c-Myc proteins. These results characterize the DEF domain in c-Myc and show that S62 phosphorylation depends on an intact DEF domain.

FIGURES 16A-F are Western blots demonstrating the kinetics of immediate early gene expression in Swiss 3T3 cells. Cells were deprived of serum growth factors and treated with EGF (25 ng/mL) or PDGF-BB (20 ng/mL) for the indicated times.

FIGURES 17A-B are Western blots demonstrating the kinetics of Egr-1, JunB, and c-Myc expression in Swiss 3T3 cells. Cells were treated as described in Figure 13. Total levels of c-Myc (Figure 17B) were detected by immunoprecipitating c-Myc prior to Western analysis.

FIGURES 18A-E are Western blots demonstrating that sustained expression of immediate early genes requires ERK1/2 activity. Serum deprived Swiss 3T3 cells were treated with PDGF-BB for 90 minutes before adding DMSO vehicle (0.1%) or UO126 (5 μ M).

FIGURE 19A is a Western blot of cells treated with PDGF-BB for either 90 minutes (lanes 2-9) or 120 minutes (lanes 11-13) before adding DMSO vehicle (lanes 3-5) or UO126 (lanes 7-9, 12, 13). FIGURE 19B is a Western blot of cells treated with PDGF-BB for 5 hours to induce Fra-1 before adding UO126 for a further 20 or 30 minutes. These figures demonstrate that ERK1/2 signaling is required during G1 for the stabilization of c-Myc.

FIGURE 20 is a series of Western blots from Swiss 3T3 cells treated with various concentrations of PDGF-BB before lysis. These results demonstrate that IEG products act as sensors for subtle differences in ERK1/2 signal duration.

FIGURE 21A is a bar graph of the result from an *in vitro* kinase assay demonstrating ERK 1/2 activation is sensitive to small differences in growth factor (PDGF) stimulation. FIGURE 21B is a Western blot demonstrating that the c-Fos stabilization observed in Figure 20 following stimulation with 10 ng/ml PDGF is a result of ERK 1/2-dependent phosphorylation of T325. Neither long-term c-Fos stabilization (see Figure 20) nor T325 phosphorylation is observed following 4 ng/ml PDGF stimulation.

FIGURE 22 are Western blots showing Fra-1 hyperphosphorylation throughout G1 requires ERK1/2 signaling.

FIGURE 23 is representative gel and the densitometric quantification of an electrophoretic mobility shift assay (EMSA) for AP-1. Swiss 3T3 cells were treated as indicated and extracted in a hypotonic lysis buffer. The nuclear fraction was isolated and aliquots mixed with a ³²P-labelled AP-1 oligonucleotide in a standard EMSA. These results demonstrate that PDGF, but not EGF, treatment significantly increases AP-1 expression and AP-1 DNA binding.

FIGURE 24 is an immunoprecipitation of extracts from 208F cells stably expressing c-Myc or c-Myc F196A and treated with cycloheximide (14 mg/mL) for the indicated times. Following immunoprecipitation of the c-Myc proteins, total levels of c-Myc were detected using Western analysis. These results demonstrate that c-Myc stability requires the DEF domain.

FIGURE 25 is a series of indirect immunofluorescence photomicrographs demonstrating typical results of the screening assays described herein. Representative fields of view using a 10X objective lens are shown.

25

Detailed Description

We have discovered that DEF domains are present in numerous proteins that are important in a variety of human diseases and, by blocking the interaction of a MAP kinase with the DEF domain of a target protein, effective therapy may be provided. Also

provided are screening methods for identifying novel therapeutics that inhibit the MAP kinase-DEF domain interaction. This invention provides several advantages over known therapies that directly target the MAP kinase signaling cascade. Typically, most compounds that inhibit the MAP kinase pathway are non-specific and inhibit more than 5 one enzyme. Also, the targeted kinases, if effectively inhibited, are not available to perform normal physiological functions necessary for cell survival, resulting in toxicity to healthy as well as diseased cells. By contrast, the therapeutic methods of the present invention inhibit the activation of particular target proteins, leaving the MAP kinases enzymatically active and available to phosphorylate other, non-DEF domain-containing 10 proteins. Diseased cells (e.g., cancerous cells) are often more susceptible to therapy because of the higher concentration of target protein.

The principles of the invention are exemplified using the immediate early gene, c-Fos, but is not intended to be limiting. c-Fos functions as a molecular sensor for the duration of extracellular-signal-regulated kinase 1/2 (ERK1/2) signaling. c-Fos is known 15 to be phosphorylated by ERK1/2 and RSK, resulting in increased stability of the protein. Therefore, the biological function of c-Fos differs under conditions where ERK1/2 signaling is sustained, rather than transient. Signaling is transduced by ERK1/2 binding to the DEF domain of c-Fos. Mutating the DEF domain inhibits c-Fos-mediated signaling and, ultimately, the downstream effects of ERK1/2 activation.

20 Further, Fos, Myc and Jun family proteins are transcription factors encoded by immediate early protooncogenes. Family members c-Fos, Fra-1, Fra-2, c-Myc, N-Myc, JunD, and JunB are frequently found to be amplified or upregulated in human cancers. Sustained ERK1/2 signaling is required for cell proliferation and ERK1/2 docking to 25 these proteins occurs only when signaling is sustained. Docking controls the growth-promoting function of these transcription factors. Accordingly, ERK1/2 docking inhibitors may be clinically useful drugs because they will likely to inhibit a specific branch of ERK1/2 signaling and would, therefore, be less toxic than general ERK1/2 inhibitors.

Sustained ERK1/2 Activation Correlates With S Phase Entry

Treatment of quiescent Swiss 3T3 fibroblasts with platelet-derived growth factor (PDGF) stimulated S phase entry; whereas, treatment with epidermal growth factor (EGF) did not (Figure 1A). The activation kinetics and amplitude of ERK1/2 and RSK, however, were almost identical following a 5-10 minute exposure to either PDGF or EGF (Figure 1B). In both cases, hyperphosphorylated (active) ERK1/2 was localized to the nucleus (Figure 1C). In contrast to ERK1/2 and RSK activation kinetics, rates of inactivation were faster in cells treated with EGF compared to PDGF (Figure 1B). 10 ERK1/2 signaling remained elevated for at least 240 minutes following PDGF exposure, but returned to basal levels within 30-45 minutes following EGF withdrawal (Figure 1B). Notably, the sustained ERK1/2 activity elicited by PDGF treatment remained localized to the nucleus (Figures 1C and 9A), demonstrating a tight correlation with S phase entry. Further, c-Fos protein expression was prolonged in cells treated with PDGF compared to 15 those treated with EGF (Figure 1D). This indicates that c-Fos becomes stabilized when ERK1/2 signaling is prolonged, but is unstable when ERK1/2 signaling is transient. c-Fos expression was not affected by either the addition of cycloheximide to cells 75 minutes after PDGF treatment (Figure 9B) or the addition of actinomycin D 20 minutes after PDGF treatment. Thus, the differences in c-Fos expression between PDGF- and 20 EGF-treated cells arises from post-translational control. This conclusion is further supported by studies showing that the transcriptional induction of *c-fos* and other IEGs by various growth factors is completed within 30-45 minutes.

ERK1/2 and RSK coordinately phosphorylate the extreme C-terminus of c-Fos at Ser 374 and Ser 362, respectively (Figure 2A). Mutating these residues to aspartate (Fos-DD), which mimics phosphorylation, results in enhanced transformation of fibroblasts by comparison to c-Fos having Ser 362 and Ser 374 mutated to alanine (Fos-AA) (Okazaki *et al.*, *EMBO J.*, 14: 5048-5059, 1995; Chen *et al.*, *Proc. Natl. Acad. Sci. USA*, 90: 10952-10956, 1993). Thus, increasing the stability of c-Fos is not the only manner in which this

transcription factor can regulate cellular transformation. Fos-AA appears to be differentially regulated compared to Fos-DD.

We have discovered that the addition of serum to fibroblasts results in a large, λ phosphatase-sensitive electrophoretic mobility shift of Fos-DD, compared to Fos-AA (Figures 2B left, and 10A). This effect correlates with increased incorporation of ³²P-orthophosphate that was consistently two to threefold greater for Fos-DD than Fos-AA (Figure 2B, right). This demonstrates that phosphorylation of Ser 362 and Ser 374 prime c-Fos for additional growth factor-regulated phosphorylation. As Fos-DD has greater transforming potential than Fos-AA, the regulation of primed c-Fos is critical for 10 promoting fibroblast proliferation.

Phosphorylation of Primed c-Fos is MEK-dependent

NIH 3T3 cells transfected with different Fos proteins were treated with the MEK inhibitor UO126 (Favata *et al.*, *J. Biol. Chem.*, 273: 18623-18632, 1998) to determine if 15 the mitogen-regulated phosphorylation of Fos-DD is mediated by the Raf/MEK/MAPK pathway. UO126 inhibited the growth factor-regulated mobility shift of Fos-WT and Fos-DD (Figure 2C) and ERK1/2 activation (Figure 2C, bottom), indicated that ERK1/2 or downstream signaling molecules regulated primed c-Fos. Identical observations were made using NIH 3T3 cells expressing a conditionally active form of B-Raf (ΔB-Raf-ER) 20 and treating these cells with tamoxifen instead of EGF (Figure 10B). To determine whether ERK1/2 could phosphorylate primed c-Fos *in vitro*, we used different hexahistidine-Fos fusion proteins as substrates. ERK1/2 efficiently phosphorylated Fos-WT (Figure 2D). The phosphorylation of Fos-AA and primed c-Fos, Fos-EE (S362E/S374E), by ERK1/2 was also easily detected *in vitro*, but the phosphorylation of 25 Fos-EE compared with Fos-AA was consistently greater (Figure 2d). These results demonstrate that ERK1/2 can phosphorylate sites in c-Fos other than Ser 374 and that this phosphorylation is enhanced after priming of the C terminus. In contrast to ERK1/2, phosphorylation of c-Fos by ERK5 *in vitro*, another UO126-sensitive proline-directed

kinase, was not observed (Figure 10C).

An ERK1/2 Targeting Motif Promotes the Phosphorylation of Primed Fos

The preference of ERK1/2 for primed c-Fos (Fos-EE) over Fos-AA demonstrates that C-terminal phosphorylation exposes additional phosphorylation sites and/or a motif that would increase the efficiency of phosphorylation at these sites. Examination of the c-Fos sequence identified a site in the C terminus that has similarity with the ERK1/2 targeting motif, FXFP (SEQ ID NO: 1), known as a DEF domain. In c-Fos, this motif is FTYP (Figure 3A; SEQ ID NO: 2). Mutating either Phe 343 or Tyr 345 to alanine dramatically inhibited the phosphorylation of primed c-Fos (Fos-EE) by ERK1/2 *in vitro* (Figure 3A). ERK1/2-regulated phosphorylation of substrates that contain DEF domains can be competitively inhibited *in vitro* with a synthetic peptide encompassing the DEF domain found in ELK-1. This peptide (FQFP; SEQ ID NO: 3) inhibited the phosphorylation of primed c-Fos in a concentration-dependent manner (Figure 3B). By contrast, a peptide with a mutant DEF domain (AQAP; SEQ ID NO: 4) was less efficient in inhibiting ERK1/2-mediated phosphorylation of primed c-Fos. The ELK-1 peptide was then engineered to contain the c-Fos FTYP DEF domain (SEQ ID NO: 2). This peptide also inhibited primed c-Fos phosphorylation, but a mutant version (ATYP; SEQ ID NO: 5) did not (Figure 3C). In both cases, the IC₅₀ for the FQFP (SEQ ID NO: 3) and FTYP (SEQ ID NO: 2) peptides was approximately 80 μM. The EGF-stimulated mobility shift of Fos-DD *in vivo* was also inhibited when Phe 343 or Tyr 345 were mutated to alanine (Figure 3D). These results demonstrate that the initial phosphorylation of c-Fos by ERK1/2 and RSK as the extreme C terminus expose an ERK1/2 docking site that allows ERK1/2 to phosphorylate additional sites.

Based on this model, mutation of Phe 343 or Tyr 345 to alanine should prevent hyperphosphorylation of Fos-WT and not interfere with the priming phosphorylations, which are C-terminal to the DEF domain. Indeed, these mutations prevented the appearance of the slowest mobility, but still allowed a shift to the intermediate mobility

(Figure 3E). This indicates that the DEF domain is not involved in directing ERK1/2 to prime c-Fos through Ser 374 phosphorylation. Instead, ERK1/2 docking through the DEF domain results in the hyperphosphorylation of primed c-Fos.

5 ERK1/2 phosphorylates Thr 325 and Thr 331 in primed c-Fos

There are two proline-directed threonine residues (Thr 325 and Thr 331) amino-terminal to the DEF domain in c-Fos (Figure 4A). Mutation of Thr 325 to alanine almost completely inhibited the phosphorylation of Fos-EE by ERK1/2 *in vitro*, and the additional mutation of Thr 331 to alanine was required to reduce the phosphorylation to background levels (Figure 4A). Thr 325 and Thr 331 were also phosphorylated in primed c-Fos (Fos-DD) *in vivo*, as evidenced by the complete loss of the mobility shift in the T325A/T331A mutant (Figure 4B). Individual mutation of Thr 325 or Thr 331 to alanine in the context of Fos-DD only partially inhibited growth factor-regulated phosphorylation. Substituting alanines for Thr 325 and Thr 331 in the context of Fos-WT prevented the EGF-stimulated to the slowest mobility (Figure 4C). However, EGF treatment was associated with the appearance of the intermediate mobility form, resulting from priming phosphorylation of ERK1/2 and RSK. Collectively, these observations demonstrate an ordered phosphorylation process whereby the initial phosphorylation of c-Fos at Ser 374 and Ser 362 (priming) exposes a DEF domain that mediates the hyperphosphorylation of c-Fos at Thr 325 and Thr 331. Further, Ser 374 phosphorylation is not regulated by docking. This is consistent with the phosphorylation of amino acids N-terminal, but not C-terminal, to DEF domains.

A phosphorylation-specific antiserum for Thr 325 in c-Fos (Figure 5A) was generated to investigate the mitogen-regulated phosphorylation of this residue in primed c-Fos. The antiserum showed little or no reactivity with Fos-WT or Fos-DD expressed in quiescent cells (Figure 5B, -EGF). However, after treatment with EGF, strong reactivity was associated with Fos-WT and Fos-DD, but not with Fos^{T325A} or Fos-DD^{T325A} (Figure 5B, +EGF). Priming of the extreme C terminus by ERK1/2 and RSK promotes additional

phosphorylation of c-Fos *in vivo* (Figure 2B). To determine if this is caused by increased phosphorylation of Thr 325, Fos-WT and Fos-AA were expressed to similar levels in the ΔB-Raf-ER NIH 3T3 cells that were then treated with tamoxifen for varying times (Figure 5C). The phosphorylation of Thr 325 was greater in cells transfected with Fos-WT than those transfected with Fos-AA.

Mutating Phe 343 or Tyr 345 to alanine prevented the hyperphosphorylation of primed c-Fos (Figure 3). Specifically, the regulation of Thr 325 phosphorylation *in vivo* was inhibited when Phe 343 or Tyr 345 were mutated to alanine, either in the context of Fos-WT (Figure 5D) or Fos-DD (Figure 5E). In this later experiment, there is a strong correlation between the mobility shift of Fos-DD and increased Thr 325 phosphorylation confirming that the DEF domain in c-Fos increases the efficiency of Thr 325 phosphorylation *in vivo*.

The phosphorylation of Thr 325 is differentially regulated by ERK1/2-signal duration

As shown above, the induction kinetics of c-Fos expression 30-45 min after addition of PDGF or EGF to Swiss 3T3 cells were identical (Figure 1D). This is consistent with a model in which an initial activation of ERK1/2 or RSK is sufficient for induction of *c-fos* IEG expression. To determine if ERK1/2 signal duration differentially regulates the phosphorylation of Thr 325 in endogenous c-Fos, we prepared extracts from Swiss 3T3 cells treated with EGF or PDGF for different times. Importantly, although c-Fos is present in cells after 45 or 60 min of EGF treatment, Thr 325 phosphorylation was not observed (Figure 6A). This is consistent with inactivation of ERK1/2 occurring before c-Fos is present (Figure 6A). By contrast, phosphorylation of Thr 325 increased 45-60 min after addition of PDGF (Figure 6B). Maximal Thr 325 phosphorylation persisted for at least 120 min (Figure 6B), but was still detected after 240 min. In Rat-1 fibroblasts, treatment with 100 μM lysophosphatidic acid (LPA) results in sustained ERK1/2 activity and S phase entry, whereas treatment with 0.1-1 μM LPA transiently

activates ERK1/2 and no cell cycle progression occurs. Although treatment of quiescent Rat-1 fibroblasts with mitogenic (100 μ M) and no-mitogenic (0.5 μ M) concentrations of LPA resulted in a similar induction of *c-fos*, phosphorylation of Thr 325 only occurred with 100 μ M LPA (Figure 11). These findings correlate with the generation of transient and sustained ERK1/2 responses by 0.5 μ M and 100 μ M LPA, respectively (Figure 11). Thus, differential phosphorylation of c-Fos occurs in different cell types and in response to agonists that directly activate tyrosine kinase receptors or heterotrimeric G protein-coupled receptors.

To show that the sustained phase of ERK1/2 signaling was required to mediate the stabilization and hyperphosphorylation of endogenous c-Fos in Swiss 3T3 cells, ERK1/2 and RSK activity was inhibited by adding UO126 to cells that had been treated with PDGF for 60 min (Figure 6D). Under this condition, the phosphorylation of Thr 325 was completely inhibited and the electrophoretic mobility of c-Fos increased (Figure 6C). Importantly, these UO126-induced changes in the biochemical properties of c-Fos also preceded the rapid disappearance of c-Fos protein. This result is consistent with hypophosphorylated c-Fos being unstable. These observations show that the phosphorylation of c-Fos at Thr 325 is tightly correlated with the activation/inactivation kinetics of ERK1/2 in different cell types and provide clear evidence that c-Fos can function as a sensor for ERK1/2 signal duration.

20

The DEF domain and Thr 325/Thr 331 phosphorylation modulates c-Fos function

In HeLa cells expressing Fos-WT, AP-1 transcription factor activity was consistently three to fourfold above background levels (Figure 7A). Mutation of Thr 325 and Thr 331 to alanine reduced AP-1 activity by about 20%; whereas, mutating the DEF domain (F343A) reduced Fos-WT activity by about 65% (Figure 7A). These observations indicate that docking of ERK1/2 to c-Fos is important in regulating c-Fos transcriptional activity under conditions of growth factor stimulation. To determine if ERK1/2 docking to c-Fos can contribute to c-Fos function independently of the

phosphorylation-mediated stabilization, Fos-WT, Fos^{T325A/T331A} or Fos^{Y345A} (a DEF domain mutant) were stably expressed in 208F fibroblasts. The expression of the different Fos proteins (Figure 7B, bottom six panels) was equivalent to the level of endogenous c-Fos in serum-stimulated vector-infected cells (Figure 7B, top two panels) and was also localized to the nucleus. Western analysis of c-Fos expression in the quiescent cell lines also showed that they were expressed to similar levels (Figure 7C). The stable expression of Fos-WT promoted anchorage-independent growth in soft agar (Figure 7D), as expected. Mutating Thr 325 and Thr 331 to alanine significantly reduced the growth of 208F cells in soft agar suggesting that phosphorylation of these residues promotes cellular transformation (Figure 7D). However, replacing Thr 325 and Thr 331 with Asp enhanced c-Fos-mediated focus formation (Figure 12). Further, mutating the c-Fos DEF domain (Fos^{Y345A}) completely inhibited the ability of c-Fos to transform 208F cells; more so than the c-Fos^{T325A/T331A} mutant (Figure 7D). These results demonstrate that ERK1/2 docking to c-Fos contributes to transformation through mechanisms in addition to Thr 325/Thr 331 phosphorylation and that stabilization of c-Fos is not the only factor that regulates c-Fos function, as all proteins were expressed equally. In addition, it also demonstrates that ERK1/2 docking to c-Fos regulates biological activity.

Mechanism of IEG Activation Through DEF Domain Binding

The mechanism described here employs an IEG product, typified by c-Fos, which functions as a molecular sensor that differentiates between differences in ERK1/2 and RSK signal duration, as well as their cytoplasmic/nuclear distribution (Figure 8). As observed for a large number of IEGs, the *c-fos* gene is transcriptionally induced within minutes of growth factor stimulation and therefore occurs with kinetics that are independent of differences in signal duration. Newly synthesized c-Fos protein has a half-life of about 30-45 min but, when phosphorylated by ERK1/2 and RSK, the half-life is extended to at least 2h. Thus, when ERK1/2 is rapidly activated (transient signal), c-Fos is present in the nucleus, but is not phosphorylated, and is therefore unstable and

degraded (Figure 8). By contrast, delayed inactivation of ERK1/2 (sustained signal) results in the efficient phosphorylation of c-Fos at its extreme C terminus, resulting in its stabilization for several hours. The initial priming phosphorylation in the C-terminus exposes a DEF domain that promotes additional ERK1/2-mediated phosphorylation

5 events, increasing the efficiency of ERK1/2-regulated phosphorylation when ERK1/2 is only sub-maximally active (0.5-4 h after stimulation). Further, when priming and docking are inhibited by point mutation (Fos-AA and Fos^{F343A} or Fos^{Y345A}, respectively) ERK1/2 and/or RSK signals are unable to alter c-Fos function. These non-phosphorylatable c-Fos mutants likely resemble the hypophosphorylated form of c-Fos that is present when

10 ERK1/2 is rapidly inactivated during transient signaling and cells do not enter S phase.

Simply prolonging the half-life of c-Fos will not affect its role in promoting transformation. Instead, the combination of protein stabilization and DEF-mediated regulation allows c-Fos to function as sensor for ERK1/2. If c-Fos is not stabilized during the sustained phase of signaling, ERK1/2 will not target the c-Fos DEF domain.

15 Therefore, stabilizing the IEG product is a critical first step if it is to function as a sensor for sustained ERK1/2 signals. The physiological importance of the c-Fos DEF domain is underscored by the fact that mutations in the DEF domain significantly reduced AP-1 activity and inhibit the transforming activity associated with wild-type c-Fos. However, the effect of mutating the DEF domain is stronger than the effect of mutating the

20 phosphorylation sites that are controlled by this docking site, indicating that the DEF domain can have more than one action. An additional function of the DEF domain ERK1/2-mediated *trans*-phosphorylation of AP-1 complex proteins.

We described a general mechanism for cellular sensing of ERK1/2 signal strength and timing involving the FTYP (SEQ ID NO: 2) DEF domain present in many IEGs.

25 Putative DEF domains are found in additional AP-1 proteins, such as Fra-1, Fra-2, Jun-B and JunD (Table 1). The proto-oncogene products c-Myc and N-Myc also contain putative DEF domains. The IEG product Egr-1 has a DEF domain and several putative proline-directed phosphorylation sites N-terminal to this domain that could enable Egr-1

to sense sustained signaling in PC12 cells and promote neuronal differentiation. In common with the c-Fos DEF domain, the other DEF domains highlighted in Table 1 show subtle deviation from the FXFP consensus (SEQ ID NO: 1), with respect to the presence of phenylalanine at positions 1 and 3 indicating that tyrosine can be tolerated at either site.

5

Table 1. DEF Domains in Immediate Early Gene Products

<i>IEG</i>	<i>Amino Acid Sequence</i>	<i>SEQ ID NO.</i>
c-Fos	³¹⁴ -GPMVTELEPLCTP-VVTCTPSCTTYTSSFV F TYPPEADS	6
Fra-1	²¹¹ -GP-VLEPEALHTPTLMT-TPSLTPFTPSLV F TYPSTPEP	7
Fra-2	¹⁶⁹ -GGFYGE-EPLHTP-IVVTSTPAITPGTSNLV F TYPSVLEQ	8
Fos-B	²⁸³ -HSEVQV-LGDPFPVV-SPS-YTSSFVLTCPEVSAF	9
JunD	⁸⁷ -LLASPDGLLKLASPELERLIIQS-NGLVTTPTST- Q FLYPKV	10
JunB	⁷¹ -GQGSDTGASLKLASSELERLIVPNSNGVITTTPTPPGQ Y FYPRG	11
c-Jun	⁶⁰ -LLTSPDVGLLKLASPELERLIIQSSNGHITTTPTPT- Q FLCPKN	12
c-Myc	⁵⁶ -LPTPPLSPSRRSGLCSPSYV ¹⁸¹ -LTA-AASECIDPSVVFPYPLND	13 14
N-Myc	⁷⁷ -AQSPGAGAASPAGRGHGGAAAGA ¹¹⁰ -AHPAAECVDPAVVFPFPVNK	15 16
Egr-1	¹⁸⁴ -QSPPLSCAVPSNDSSPIYSAAPTFTPTPNTD ²⁴⁷ -PMIPDYLFPQQ	17 18
MPer1	⁶⁹⁷ -PRGGPQPLPPAPTSVPPAAFPAVLVTPMVALPNYL F PTPSSY	19
DEF domains are in bold and number indicate amino acid position. Sequences are from rat (c-Fos, Fra-1, and Fra-2), mouse (FosB, JunD, c-Jun, c-Myc, and Egr-1), or human (JunB, N-Myc, and mPer1).		

Screening Methods to Identify Inhibitors of DEF Domain Binding

DEF Domain Binding Assessment using a Phospho-specific Antibody

We have developed a cell-based assay which is used to screen small molecule compound libraries (Figure 25). In this assay, rat 208F fibroblasts that stably express 5 c-Fos are cultured in a 384-well plate and deprived of serum growth factors for 24 hours. Cells are then treated with EGF for 15 minutes and fixed with 3.7% formaldehyde. Permeabilized fixed cells are incubated with DAPI to stain the nuclei and an anti-phospho-ERK1/2 mouse monoclonal antibody and an anti-phospho-T325 Fos rabbit polyclonal antibody for 2 hours. Anti-mouse Alexa594-conjugated IgG and an anti-rabbit 10 FITC-conjugated IgG are added to each well and unbound antibody is removed by several washes. The fluorescence intensity of both fluorophores in each well can be detected using an automated epifluorescence microscope or Autoscope (Universal Imaging Systems, Inc.). A clear increase in T325 phosphorylation was observed in cells treated with EGF (indicating that ERK1/2 docking to Fos has taken place). In the same 15 population of cells, the phosphorylation of ERK1/2 also increased, thus indicating its activation by EGF. Under these conditions, only background levels of fluorescence were detected when both phospho-specific antibodies were omitted, and the secondary antibodies show no cross-species reactivity.

Inhibition of ERK1/2 docking to the c-Fos DEF domain *in vivo* could result from 20 compounds that (a) are generally toxic, (b) prevent the activation of ERK1/2, (c) prevent the growth factor regulated translocation of ERK1/2 into the nucleus where c-Fos is localized or (d) directly antagonize ERK1/2 docking to the DEF domain. The assay we have developed naturally excludes the first three possibilities. First, toxicity will be reflected by nuclear integrity as visualized with DAPI staining. Second, inhibition of 25 ERK1/2 activation/activity will be apparent from the phospho-ERK1/2 fluorescence signal. Third, nuclear translocation of ERK1/2 can be verified by manually examining images from wells that show decreased c-Fos phosphorylation. Therefore, candidate compounds that specifically inhibit ERK1/2 binding to the DEF domain are defined as

compounds that decrease the phosphorylation of T325 in c-Fos but which have no effect on ERK1/2 activation or its localization. Although this assay is exemplified using rat fibroblasts, it may be performed using any appropriate cell type including, for example, myoblasts, epithelial cells, and hepatocytes.

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Interaction-Trap Assays

A standard yeast two-hybrid assay may be used to assess the effect of a test compound on the MAP kinase-DEF domain interaction (Mendelsohn and Brent, *Curr. Opin. Biotechnol.* 5:482-486, 1994). Typically, a vector encoding a synthetic or naturally occurring peptide containing a DEF domain, covalently bound a DNA binding domain (e.g., GAL4), is transfected into yeast cells containing a reporter gene operably linked to a binding site for the DNA binding domain. Further, a vector encoding either the native MAP kinase of interest, or a synthetic fragment containing the sequence that interacts with the target DEF domain, covalently bound to a transcriptional activator (e.g., GalAD) is also transfected. The effectiveness of a test compound is then assessed by growing the yeast in the presence of the compound and measuring the level of reporter gene expression.

GST Pulldown Assays

20 The interaction of a MAP kinase with a DEF domain may be examined using a GST-fusion protein binding study. A vector encoding a naturally-occurring or synthetic polypeptide containing the DEF domain of interest is fused to GST and expressed in a host cell (e.g., *E. coli* or *Saccharomyces spp.*). The GST fusion protein is then contacted with a MAP kinase in the presence and absence of a test compound. The MAP kinase 25 may be naturally expressed by the host cell or may be expressed from a second vector inserted into the host cell. Following incubation with the test compound, the host cells are lysed and the GST fusion proteins are recovered using glutathione-Sepharose (GSH-Seph) beads. Typically, the GST fusion proteins are released from the GSH-Seph by

boiling and the proteins visualized by electrophoretic separation on an SDS-PAGE gel. A skilled artisan will readily understand that the GST-Pulldown assay described here can be readily adapted to a cell-free assay by incubating the purified GST fusion protein with a purified recombinant MAP kinase.

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Fluorescence Polarization Assay

A variety of well known cell-free techniques may be used to assess the effects of a test compound on the interaction between a MAP kinase and a DEF domain-containing target protein. Fluorescence polarization assays are particularly useful for this purpose.

10 In this assay, a peptide (about 6-12 amino acids) containing a DEF domain at its C-terminus and a fluorophore (e.g., fluorescein) conjugated to its N-terminus is incubated in the presence and absence of increasing amounts of recombinant MAP kinase (e.g., GST-ERK1; 0.01-1 μ M) for 10 minutes at room temperature. Aliquots from each reaction are placed in a plate black-walled microtiter (e.g., 384-well) plate and polarization measured
15 using an Analyst plate reader. Increasing concentrations of the MAP kinase causes an increase in polarization. Titrating in the "free" DEF domain-containing peptide (i.e., unconjugated) inhibits the change in polarization, whereas the mutated DEF domain peptide does not. The appearance of low polarization, even in the presence of high concentrations of kinase, indicates flexible binding of the DEF domain to the kinase and
20 suggests the presence of the propeller effect. Designing shorter dye-conjugated DEF domain-containing peptides usually alleviates this problem. The effect of standard assay variables, including incubation time, temperature, pH (7.2-8.5), and buffers, on polarization is readily controlled during routine assay optimization.

This assay is readily adaptable for identifying test compounds that inhibit binding

25 of a MAP kinase to a DEF domain. The use of automated liquid handling systems and plate readers makes this assay readily adaptable to a high-throughput format for screening large numbers of test compounds. For compound screening, the test compound is added to a mixture of the fluorescently labeled DEF domain-containing peptide and the target

MAP kinase. Compounds that inhibit the polarization increase (or cause a decrease in polarization) resulting from increasing amounts of the MAP kinase are therapeutic candidates.

5 *Identification of Test Compounds as Potential Therapeutics*

We have identified a variety of DEF domain-containing target proteins that have been implicated in a variety of diseases. The particular DEF domain or target protein may be substituted for c-Fos in any of the exemplary assays described here. Further, the lists of target proteins provided are not intended to be limiting. Other target proteins are easily 10 identified based on the availability of a DEF domain.

Test compounds having antineoplastic activity are those that inhibit binding of a MAP kinase (e.g., ERK 1/2) to the DEF domain of any of the proteins of Table 1 (except mPer1) or Table 2. Test compounds that are useful for treating cardiovascular disorders inhibit MAP kinase binding to the DEF domain of the proteins identified in Table 3. Test 15 compounds that are useful for treating acute and chronic inflammation or inflammatory disorders inhibit MAP kinase binding to the DEF domain of the proteins identified in Table 4. Test compounds that are useful for treating a variety of metabolic disorders inhibit MAP kinase binding to the DEF domain of the proteins identified in Table 5. Test compounds that are useful for treating a variety of nervous system disorders (e.g., central 20 and peripheral neuropathies) and behavioral disorders (e.g., psychosis, schizophrenia, autism, Down's Syndrome, Parkinson's Disease, Alzheimer's Disease, epilepsy, Cockayne syndrome, depression, and opiate addiction) inhibit MAP kinase binding to the DEF domain of the proteins identified in Table 6. Test compounds useful for treating sleep disorders inhibit MAP kinase binding to the DEF domain of the Per proteins (Table 25 1: mPer; Table 7). Other potentially useful therapeutics inhibit MAP kinase binding to the DEF domain of the PKA-anchoring proteins (AKAPs) (Table 7). In each of Tables 2-7, the alpha-numeric Accession Codes refer to the SWISS-PROT accession numbers. The numeric Accession Codes refer to the GENPEPT accession numbers. In each case,

the DEF domain is underscored.

Administration of a DEF Domain Inhibitors or Candidate Compounds for the Treatment of Disease

5 As described above, ERK1/2 activate several IEG products through an interaction with the DEF domain and a subsequent phosphorylation event. It is also well known that activation of certain IEGs, and the proteins identified in Table 2, cause cellular proliferation and may cause tumor promotion and progression. Accordingly, this invention also provides methods and compositions for antineoplastic (i.e., cancer) therapy

10 by administering DEF domain inhibitors. Likewise, therapy for cardiovascular disorders, inflammatory disorders, metabolic disorders, neuropathies and behavioral disorders, and sleep disorders may be provided by inhibiting MAP kinase binding to the DEF domain of one or more of the proteins identified in Table 3, 4, 5, 6, and 7, respectively. Useful DEF domain inhibitors include compounds that bind to the DEF domain of target proteins and

15 prevent the binding of the target kinases. Also, DEF domain inhibitors include "bait" proteins that bind activated target kinases but do not cause cellular proliferation or tumor promotion and/or progression.

In addition to candidate compounds identified using the screening methods of this invention, DEF domain inhibitors can be created by inserting, by artifice, a DEF domain into a non-target protein. The cellular activation/proliferation pathway described herein is limited by the presence of activated target kinase, not by the availability of target proteins. Thus, a DEF domain that is present in a non-target protein effectively "baits" the target kinase, reducing its availability to phosphorylate the target proteins. DEF domains suitable for therapy have the general structure: F/Y—X₁—F/Y—X₂ (SEQ ID NO: 28).

25 Desirably, X₂ is proline. Most desirably, the DEF domain is identical to the DEF domain of the target protein to which therapy is directed. For example, Figures 3B and C demonstrate that the "naked" DEF domains FQFP (SEQ ID NO: 3) and FTYP (SEQ ID NO: 2) are effective inhibitors of target protein phosphorylation. Substitution of

phenylalanine for alanine in these polypeptides results in approximately a two-fold reduction in potency. Accordingly, therapy can be provided by administering pharmaceutical formulations containing a naked DEF domain. Typically, these polypeptides are administered by parenteral injection such as intravenous, intramuscular, 5 or subcutaneous injection. These small polypeptides may be administered in any appropriate formulation including, for example, in a liposomal formulation. The polypeptides may also be injected directly into a solid tumor.

Alternatively, therapy can be achieved by administering a chimeric protein consisting of a DEF domain that is engineered into a non-target protein. Typically, the 10 chimeric protein will "display" the four amino acid DEF domain on a hydrophilic face, making it available to bind to the activated target kinase. The non-target protein can be chosen based upon the desired pharmacokinetic or pharmacodynamic effect and is readily determined by a person of ordinary skill. For example, a DEF domain inhibitor sequence may be engineered into a serum protein such as albumin or ceruloplasmin in order to 15 prolong the plasma half life. Alternatively, the DEF domain may be engineered into a protein that promotes uptake into a particular cell type.

Pharmaceutical Formulations

The peptide agents and candidate compounds of the invention can be administered 20 to a subject, e.g., a human, directly or in combination with any pharmaceutically acceptable carrier or salt known in the art. Pharmaceutically acceptable salts may include non-toxic acid addition salts or metal complexes that are commonly used in the pharmaceutical industry. Examples of acid addition salts include organic acids such as acetic, lactic, pamoic, maleic, citric, malic, ascorbic, succinic, benzoic, palmitic, suberic, 25 salicylic, tartaric, methanesulfonic, toluenesulfonic, or trifluoroacetic acids or the like; polymeric acids such as tannic acid, carboxymethyl cellulose, or the like; and inorganic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid phosphoric acid, or the like. Metal complexes include zinc, iron, and the like. One exemplary pharmaceutically

acceptable carrier is physiological saline. Other physiologically acceptable carriers and their formulations are known to one skilled in the art and described, for example, in Remington's Pharmaceutical Sciences, (19th edition), ed. A. Gennaro, 1995, Mack Publishing Company, Easton, PA.

5 Pharmaceutical formulations of a therapeutically effective amount of a peptide agent or candidate compound of the invention, or pharmaceutically acceptable salt-thereof, can be administered orally, parenterally (e.g. intramuscular, intraperitoneal, intravenous, or subcutaneous injection), or by intrathecal or intracerebroventricular injection in an admixture with a pharmaceutically acceptable carrier adapted for the route
10 of administration.

Methods well known in the art for making formulations are found, for example, in Remington's Pharmaceutical Sciences (19th edition), ed. A. Gennaro, 1995, Mack Publishing Company, Easton, PA. Compositions intended for oral use may be prepared in solid or liquid forms according to any method known to the art for the manufacture of
15 pharmaceutical compositions. The compositions may optionally contain sweetening, flavoring, coloring, perfuming, and/or preserving agents in order to provide a more palatable preparation. Solid dosage forms for oral administration include capsules, tablets, pills, powders, and granules. In such solid forms, the active compound is admixed with at least one inert pharmaceutically acceptable carrier or excipient. These
20 may include, for example, inert diluents, such as calcium carbonate, sodium carbonate, lactose, sucrose, starch, calcium phosphate, sodium phosphate, or kaolin. Binding agents, buffering agents, and/or lubricating agents (e.g., magnesium stearate) may also be used. Tablets and pills can additionally be prepared with enteric coatings.

Liquid dosage forms for oral administration include pharmaceutically acceptable
25 emulsions, solutions, suspensions, syrups, and soft gelatin capsules. These forms contain inert diluents commonly used in the art, such as water or an oil medium. Besides such inert diluents, compositions can also include adjuvants, such as wetting agents, emulsifying agents, and suspending agents.

Formulations for parenteral administration (i.e., intravenous, intramuscular, and subcutaneous injection) include sterile aqueous or non-aqueous solutions, suspensions, or emulsions. Examples of suitable vehicles include propylene glycol, polyethylene glycol, vegetable oils, gelatin, hydrogenated naphthalenes, and injectable organic esters, such as 5 ethyl oleate. Such formulations may also contain adjuvants, such as preserving, wetting, emulsifying, and dispersing agents. Biocompatible, biodegradable lactide polymer, lactide/glycolide copolymer, or polyoxyethylene-polyoxypropylene copolymers may be used to control the release of the compounds. Other potentially useful parenteral delivery systems for the proteins of the invention include ethylene-vinyl acetate copolymer 10 particles, osmotic pumps, implantable infusion systems, and liposomes.

Liquid formulations can be sterilized by, for example, filtration through a bacteria-retaining filter, by incorporating sterilizing agents into the compositions, or by irradiating or heating the compositions. Alternatively, they can also be manufactured in the form of 15 sterile, solid compositions which can be dissolved in sterile water or some other sterile injectable medium immediately before use.

The amount of active ingredient in the compositions of the invention can be varied. One skilled in the art will appreciate that the exact individual dosages may be adjusted somewhat depending upon a variety of factors, including the protein being administered, the time of administration, the route of administration, the nature of the formulation, the 20 rate of excretion, the nature of the subject's conditions, and the age, weight, health, and gender of the patient. Generally, dosage levels of between 0.1 $\mu\text{g}/\text{kg}$ to 100 mg/kg of body weight are administered daily as a single dose or divided into multiple doses. Desirably, the general dosage range is between 250 $\mu\text{g}/\text{kg}$ to 5.0 mg/kg of body weight 25 per day. Wide variations in the needed dosage are to be expected in view of the differing efficiencies of the various routes of administration. For instance, oral administration generally would be expected to require higher dosage levels than administration by intravenous injection. Variations in these dosage levels can be adjusted using standard empirical routines for optimization, which are well known in the art. In general, the

precise therapeutically effective dosage will be determined by the attending physician in consideration of the above identified factors.

The protein or candidate compound of the invention can be administered in a sustained release composition, such as those described in, for example, U.S. Patent No.

5. 5,672,659 and U.S. Patent No. 5,595,760. The use of immediate or sustained release compositions depends on the type of condition being treated and the desired pharmacokinetic profile. For preventive or long-term treatments, a sustained released composition may be preferred.

The protein or candidate compound of the present invention can be prepared in any 10 suitable manner. The protein or candidate compound can be isolated from naturally occurring sources, recombinantly produced, or produced synthetically, or produced by a combination of these methods. The synthesis of short peptides is well known in the art. See e.g. Stewart et al., *Solid Phase Peptide Synthesis* (Pierce Chemical Co., 2d ed., 1984).

15 Methods

Cell Culture

NIH 3T3 fibroblasts were transfected with Lipofectamine (Invitrogen, Carlsbad, CA) and then cultured for 18 h in DMEM/10% calf serum. Swiss 3T3 fibroblasts expressing a conditionally active form of B-Raf, were cultured in DMEM containing 10% 20 fetal bovine serum (FBS). Before stimulation, cells were cultured in DMEM containing 20 mM HEPES (starving medium) for 24 h (NIH 3T3 or 208F) or 48 h (Swiss 3T3). Rat-1 fibroblasts were cultured for 48 h, washed with starving medium and culture for an additional 24 h in starving medium. For AP-1 assays, Hela cells were transfected with 25 Lipofectamine for 6 h and then cultured for an additional 16 h before cell lysis and assay of luciferase activity (Promega). EGF and PDGF (Invitrogen) were reconstituted in sterile water containing 0.1% BSA. LPA (Aventi Polar Lipids, Alabaster, AL) was reconstituted in 50% ethanol before sonication for 30 min.

Retroviruses used to infect rat 208F cells were produced as described previously

(Chen *et al.*, *Oncogene*, 12:1493-1502, 1996). Neomycin-resistant pools of c-Fos-expressing cells were assayed for anchorage-independent growth or focus formation. Metabolic labeling with ³⁵S-methionine or ³²P-orthophosphate (performed in parallel) was performed as described by Chen *et al.*

5

Cell lysis and western analysis

Cell extracts were prepared as described previously (Richards, *et al.*, *Curr. Biol.*, 9: 810-820, 1999). To analyze shifts in c-Fos mobility, samples were resolved on a 7.5% SDS-polyacrylamide gel electrophoresis (PAGE) gel, transferred to nitrocellulose and probed with anti-c-Fos antibody (Update Biotechnology Inc., Lake Placid, NY). This antibody is specific for c-Fos and does not cross-react with FosB, Fra-1 or Fra-2. For ERK1/2-MAPK western analysis, a polyclonal anti-ERK1/2 antibody to an anti-phospho-p42/p44 MAPK monoclonal antibody was used (Sigma, St. Louis, MO). Phosphatase treatment of cell extract was performed for 30 min on ice using λ protein phosphatase (New England Biolabs, Beverly, MA).

To generate antiserum specific for phosphorylated Thr 325 in c-Fos, residues 317-329 (VTELEPLCTPVVT) (SEQ ID NO: 20) were synthesized (underlined residue is phospho-Thr at position 325), conjugated to keyhole limpet haemocyanin and injected into rabbits (Research Genetics, Inc., Huntsville, AL). To determine the specificity of the antiserum, extracts from cells expressing vector or c-Fos proteins were immobilized on nitrocellulose and probed with a solution of this anti-serum (1:3000) for 12 h at 4°C.

Recombinant protein purification

M15pREP4 cells transformed with pDS56-(His)₆Fos or pETHis₆/ERK2 and MEK 25 1 R4F were cultured at 25°C until an OD₆₀₀ of 0.7 was attained. Cells were then incubated in the presence of 1mM isopropyl- β -D-thiogalactoside (IPTG) for an additional 12 h at 25 ° C. and then harvested by centrifugation. Pellets were resuspended in column buffer (20 mM Tris-HCl at pH 8.0, 200 mM sodium chloride, 10% glycerol, and 10 mM

imidazole) and cells were lysed by passage through a French Press. The (His)₆ proteins were purified using Nickel-NTA-agarose resin (Qiagen, Alencia, CA), dialyzed in column buffer containing 50% glycerol and then stored at -20°C.

5 ***In vitro* kinase reactions**

Phosphorylation of (His)₆-Fos by ERK1 immunoprecipitated from NIH3T3 cells, activate (His)₆-ERK2 (ref. 42) or FLAG-ERK5/BMK1, was performed in kinase buffer containing 10 μ Ci γ^{32} P-ATP at 30°C (Chen *et al.*, *Mol. Cell. Biol.* 10:3204-3215, 1990). Endogenous ERK1 and RSK kinase activities were performed as described previously 10 (Chung *et al.*, *Mol. Cell. Biol.*, 11:1868-1871, 1991). The HPLC-purified synthetic peptides used in the competition kinase assays were mixed with (His)₆-Fos-EE before addition of activated ERK2 and γ^{32} P-ATP. The peptides derived from ELK-1 were as follows: RRPRSPA^KL^SFQFP^SFQFP (SEQ ID NO: 21); RRPRSPA^KL^SAQAPSAQAP (SEQ ID NO: 22); RRPRSPA^KL^STYPSFTY^P (SEQ ID NO: 23); 15 RRPRSPA^KL^STYPSATY^P (SEQ ID NO: 24).

Immunofluorescence

Swiss 3T3 cells (1.35×10^5 per 35-mm dish) were cultured on poly-L-lysine-coated glass coverslips for 24 h and serum-starved for 48 h. Cyclohexamide (14 μ g/ml) 20 was delivered in dimethyl sulphoxide. After stimulation with growth factors, cells were washed with ice-cold PBS containing 0.1% BSA, fixed with 3.7% formaldehyde for 10 min at room temperature and permeabilized with 0.2% Triton-X100 for 5 min. Analysis of c-Fos expression was performed using a rabbit anti-human c-Fos antibody (1:500, Upstate Biotechnology Inc.) under conditions described by the manufacturer. Conditions 25 for phospho-p24/p44 MAPK immunofluorescence were identical to those used for c-Fos, except that a monoclonal phospho-MAPK antibody was used (Sigma). Coverslips were mounted in Citifluor (Ted Pella Inc., Redding, CA) and examined under epifluorescent illumination.

Bromodeoxyuridine (BrdU) incorporation

Swiss 3T3 cells were cultured as described for immunofluorescence studies, treated with growth factors and 20 μ M BrdU Labeling Reagent (Amersham Life Sciences Inc., Piscataway, NJ) for 20 h at 37 °C. For immunofluorescence analysis, a mouse anti-BrdU monoclonal (Amersham Life Sciences Inc.) supplemented with DNAase I (Invitrogen) was used.

Other Embodiments

10 All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference. Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those of ordinary 15 skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

What is claimed is:

TABLE 2: ONCOLOGY

Accession Cod	Target Description	Amino Acid	Targ t S qu nc
1 PIP3_HUMAN	1-phosphatidylinositol-4,5-bisphosphate phosphodiesterase beta 3 (EC 3.1.4.11) (Phosphoinositide phospholipase C) (PLC-beta-3) (Phospholipase C-beta-3). 26S proteasome non-ATPase regulatory subunit 1 (26S proteasome regulatory subunit S1) (26S proteasome subunit p112).	777 DEEPEDEPKVVLPLTL	
2 PSD1_HUMAN	26S proteasome non-ATPase regulatory subunit 1 (26S proteasome regulatory subunit S1) (26S proteasome subunit p112).	767 TQFWFWFPLSHFLSL	
3 PSD1_HUMAN	28S ribosomal protein S31, mitochondrial precursor (S31mt) (MRP-S31) (Imogen 38).	807 KPSTFAYPAPLEVPK	
4 RT31_HUMAN	3-phosphoinositide dependent protein kinase-1 (EC 2.7.1.37) (hPDK1).	307 EGKLWEFPINNEAGF	
5 PDPK_HUMAN	40S ribosomal protein S3.	295 IKLEYDFPEKFFPKA	
6 RS3_HUMAN	52 kDa repressor of the inhibitor of the protein kinase (p58IPK-interacting protein) (58 kDa interferon-induced protein kinase-interacting protein) (P58IPK) (Death associated protein 4). 53 kDa BRG1-associated factor A (Actin-related protein Baf53a) (ApNbeta).	73 VQKRGFPEGSVELY	
7 P52K_HUMAN	5'-AMP-activated protein kinase, catalytic alpha-1 chain (EC 2.7.1.-) (AMPK alpha-1 chain).	18 DLAFFERFPDPARCQ	
8 B53A_HUMAN	5'-AMP-activated protein kinase, catalytic alpha-2 chain (EC 2.7.1.-) (AMPK alpha-2 chain).	283 PTVHYEFPNNGNCDF	
9 AAK1_HUMAN	60S acidic ribosomal protein P0 (L10E).	273 DLPKYLFPEPDPSYSS	
10 AAK2_HUMAN	60S ribosomal protein L10 (QM protein) (Tumor suppressor QM) (Laminin receptor homolog).	271 DLPSYLFPEPDPSYDA	
11 RLA0_HUMAN	80 kDa MCM3-associated protein (GANP protein).	253 VETDYTFFPLAEKVKA	
12 RL10_HUMAN	Achaete-scute homolog 3 (bHLH transcriptional regulator Sgn-1) (Mash- 3).	152 RRAFKFPPGRQKIH	
13 MC3A_MOUSE	80 kDa MCM3-associated protein (GANP protein).	184 GLTPPESEFPQVTNNSV	
14 ASH3_MOUSE	Achaete-scute homolog 3 (bHLH transcriptional regulator Sgn-1).	69 DPYPFPFPMPYPTNYR	
15 ASH3_HUMAN	ADAM 10 precursor (EC 3.4.24.-) (A disintegrin and metalloproteinase domain 10) (Mammalian disintegrin-metalloprotease) (Kuzbanian protein homolog).	69 EPCPFSFPPMPYPPNRY	
16 AD10_HUMAN	ADAM 12 precursor (EC 3.4.24.-) (A disintegrin and metalloproteinase domain 12) (Melintrin alpha).	286 PTNPFRFPNIQGVEKF	
17 AD12_HUMAN	Adenovirus 5 E1A-binding protein (BS69 protein).	382 ASTGYPFPMVFSSCS	
18 BS69_HUMAN	Adenylate cyclase, type IV (EC 4.6.1.1) (ATP pyrophosphate-lyase) (Adenyl cyclase).	229 PDNWFCYPCIPCNHEL	
19 CYA4_HUMAN	AF-4 protein (Proto-oncogene AF4) (FEL protein).	679 ITSUFFFPPTSSDCPF	
20 AF4_HUMAN	A-kinase anchor protein 11 (Protein kinase A anchoring protein 11) (PRKA11) (A kinase anchor protein 220 kDa) (AKAP 220) (hAKAP220).	381 EPSKFFFPPTKDSQHV	
21 AK11_HUMAN	A-kinase anchor protein 3 (Protein kinase A anchoring protein 3) (PRKA3) (A-kinase anchor protein 110 kDa) (AKAP 110) (Sperm oocyte binding protein) (Fibrous sheath protein of 95 kDa) (FSP95).	661 EVCOQFSYQPQTTPASPK	
22 AKA3_HUMAN	ALK tyrosine kinase receptor precursor (EC 2.7.1.112) (Anaplastic lymphoma kinase) (CD246 antigen).	490 SDISFEYPEDIGNLs	
23 ALK_HUMAN	Ankyrin repeat domain protein 5.	264 LECSFDFPCELEYSP	
24 ANR5_HUMAN	Alkyl toxin receptor precursor (Tumor endothelial marker 8).	437 VIPEYAFFPRRQDGGP	
25 ATR_HUMAN	Anthrax toxin receptor precursor (Tumor endothelial marker 8).	421 PEQEYEFPPEPRNLNN	
26 KRAA_HUMAN	A-Raf proto-oncogene serine/threonine-protein kinase (EC 2.7.1.-) (A-ref-1) (Proto-oncogene Pks).	193 DPEHFPFPAPANAPL	

27	ABC3_HUMAN	ATP-binding cassette, sub-family A, member 3 (ATP-binding cassette transporter 3) (ATP-binding cassette 3) (ABC-C transporter).	65	LPLFFFT PPP PGDTWE
28	KU70_HUMAN	ATP-dependent DNA helicase II, 70 kDa subunit (Lupus Ku autoantigen protein p70) (Ku70) (70 kDa subunit of Ku antigen) (Thyroid-lupus autoantigen) (TLAA) (CTC box binding factor 75 kDa subunit) (CTCBF) (CTC75).	362	RPSL F VY P EE S L V G
29	AXU1_HUMAN	AXIN1 up-regulated gene 1 protein (TGF-beta induced apoptosis protein 3) (TAIP-3) (URAX1 protein).	88	GITV EY FP R CQGFTS
30		12751139 B aggressive lymphoma long isoform [Homo sapiens]	240	SSG I F Q FP L NL C T K T
31		11544936 bA271B5.1 (similar to ribosomal protein S7) [Homo sapiens]	183	KDVNF E F P E F QL Q L Q T K
32	ACTY_HUMAN	Beta-centractin (Actin-related protein 1B) (ARP1B).	29	QIPK Y CF P NY V GRPK
33	BRC1_HUMAN	Breast cancer type 1 susceptibility protein.	825	DTEG G K Y PLGHEVN H
		Bromodomain adjacent to zinc finger domain protein 1A (ATP-utilizing chromatin assembly and remodeling factor 1) (hACF1) (ATP-dependent chromatin remodelling protein) (Williams syndrome transcription factor-related chromatin remodeling factor 180) (WCRF18)	254	QDFSY Y FP D DP P P T FI
34	BA1A_HUMAN	calcium and DAG-regulated guanine nucleotide exchange factor II [Homo sapiens]	627	EEGP F FP N GEA E H
35		Calcium/calmodulin-dépendent protein kinase type II alpha chain (EC 2.7.1.123) (CaM-kinase II)	226	KAGAY D FP S PEW D TV
36	KCCA_HUMAN	CASP8 and FADD-like apoptosis regulator precursor (Cellular FLICE-like inhibitory protein) (c-FLIP) (Caspase-eight-related protein) (Casper) (Caspase-like apoptosis regulatory protein) (CLARP)	282	HIQL F LP K SHD I T Q
37	CFLA_MOUSE	(MACH-related inducer of toxicity) (MRICT) (Caspase homolog) (C)	98	EALEF Y Y P E H F T LL T
38	CARA_HUMAN	Caspase recruitment domain protein 10 (CARD-containing MAGUK protein 3) (Carma 3).	86	ESLEF Y Y P E L Y K L V T
39	CARB_MOUSE	Caspase recruitment domain protein 11 (CARD-containing MAGUK protein 3) (Carma 1).		
40	CARF_HUMAN	Caspase recruitment domain protein 15 (Nod2 protein) (Inflammatory bowel disease protein 1).	323	QEFLFV F PF S CRQLQ
41	CAR6_HUMAN	Caspase recruitment domain protein 6.	728	LENS W LF P TRIGGN F
42	CTD1_HUMAN	Catenin delta-1 (p120 catenin) (p120(ctn)) (Cadherin-associated Src substrate) (CAS) (p120(cas)) (Catenin delta-2 (Delta-catenin) (Neural plakophilin-related ARM-repeat protein) (NPRAP) (Neurojungin) (GT24).	202	LPRN H Y P PD G YSRH
43	CTD2_HUMAN	CCAAT/enhancer binding protein alpha (C/EBP alpha).	162	PEGSEQ Y PAS Y HSNQ
44	CEBA_HUMAN	CCAAT/enhancer binding protein alpha (C/EBP alpha).	27	SSAAFG F PRGAGPAQ
45	CEBA_HUMAN	CCAAT/enhancer binding protein epsilon (C/EBP epsilon).	102	GGGDFD P PGAPAGPG
46	CEBE_HUMAN	Cdc42 GTPase-activating protein [Mus musculus]	84	DPRFAY P PH T FGPD
47		Cdc42 guanine nucleotide exchange factor zizimin 1.	673	SPAPEPE P AGSLP
48	ZIZ1_HUMAN	Cdk5 and abl enzyme substrate 2 (interactor with cdk3 2) (Ik3-2).	1457	RSLIY K FP S TFY E GR
49	CBL2_HUMAN	Clathrin coat assembly protein AP50 (Clathrin coat associated protein AP50) (Plasma membrane adaptor AP-2 50 kDa protein) (HA2 50 kDa subunit) (Clathrin assembly protein complex 2 medium chain) (AP-2 mu 2 chain).	241	SYAKFLY P TNALV T H
50	A2M1_HUMAN	Contactin associated protein-like 3 precursor (Cell recognition molecule Caspr3).	114	EILD F GY P QNSETGA
51	CTA3_HUMAN	Contactin associated protein-like 3 precursor (Cell recognition molecule Caspr3).	126	EESIW G FP G NTNADS
52	CRKL_HUMAN	Crk-like protein.	128	VRTLY D FP G NDAEDL

53 CRN1_HUMAN	Crooked neck-like protein 1 (Crooked neck homolog) (hCm) (CGI-201) (MSTP021).	800 EYFDYI F PEDAANQP
54 113856444	CTCL tumor antigen se2-1 [Homo sapiens]	42 TEDD EE FPFAKTNLS
55 SRA4_RAT	CTD-binding SR-like protein RA4 (Fragment).	158 PQAP G YPG D GMQQP
56 CG1C_HUMAN	Cyclin C.	138 TRFSY A FP K EFPYRM
57 CCT1_HUMAN	Cyclin T1 (Cyclin T) (CycT1).	598 SSLNFS F PSLPTMGQ
58 CPAC_MOUSE	Cytochrome P450 2A12 (EC 1.14.14.1) (CYP11A12) (Steroid hormones 7- alpha-hydroxylase) (Testosterone 7-alpha-hydroxylase).	456 QNFREK F PRKLEDIN
59 BMX_HUMAN	Cytoplasmic tyrosine-protein kinase BMX (EC 2.7.1.112) (Bone marrow kinase BMX) (Epithelial and endothelial tyrosine kinase) (ETK) (NTK38).	273 SKISW E FPESSSEE
60 PA24_HUMAN	Cytosolic phospholipase A2 (CPLA2) [Includes: Phospholipase A2 (EC 3.1.1.4) (Phosphatidylcholine 2-acylhydrolase); Lysophospholipase (EC 3.1.1.5)].	679 STNFQ Y PNQAFKRL
61 5NTC_HUMAN	Cytosolic purine 5'-nucleotidase (EC 3.1.3.5) (5'-nucleotidase cytosolic II).	260 MTYLF D FP H GP K PGS
62 1616601	disintegrin-metalloprotease MADM [Homo sapiens]	229 PTNPF R FP N ISVEKF
63 DM3A_HUMAN	DNA (cytosine-5)-methyltransferase 3A (EC 2.1.1.37) (Dnmt3a) (DNA methyltransferase HsallIA) (DNA MTase HsallIA) (M.HsallIA).	861 MERV G FPVHYTDVS
64 DM3B_HUMAN	DNA (cytosine-5)-methyltransferase 3B (EC 2.1.1.37) (Dnmt3b) (DNA methyltransferase HsallIB) (DNA MTase HsallIB) (M.HsallIB).	805 LERIF G FPVHYTDVS
65 DNM2_HUMAN	DNA mismatch repair protein 2 (Dnm2) (DNA methyltransferase homolog HsallP) (M.HsallP) (PuMeI).	354 FPPEFG F PEK I TVKQ
66 MLH3_HUMAN	DNA mismatch repair protein Mlh3 (MutL protein homolog 3).	1285 LGLEF V FPDTSDSLV
67 DPD2_HUMAN	DNA polymerase delta subunit 2 (EC 2.7.7.7).	389 KTD P IF E CPHVVYF
68 DPE2_HUMAN	DNA polymerase epsilon subunit B (EC 2.7.7.7) (DNA polymerase II subunit B).	236 HVNA G FP T EPSSST
69 DPG2_HUMAN	DNA polymerase gamma subunit 2, mitochondrial precursor (EC 2.7.7.7) (Mitochondrial DNA polymerase accessory subunit) (PolG-beta) (MtpolB) (DNA polymerase gamma accessory 55 kDa subunit) (p55).	287 NKLYYNFPWG K ELIE
70 TP2A_MOUSE	DNA topoisomerase II, alpha isozyme (EC 5.99.1.3).	633 HRIQ K Y P GP D DAA
71 TP2B_HUMAN	DNA topoisomerase II, beta isozyme (EC 5.99.1.3).	1444 FGNL F SP S Y S Q K SE
72 STAU_HUMAN	Double-stranded RNA-binding protein Staufen homolog.	114 PRY F YP V P P LLYQ
73 MPK4_HUMAN	Dual specificity mitogen-activated protein kinase kinase 4 (EC 2.7.1.-) (MAP kinase kinase 4) (JNK activating kinase 1) (c-Jun N-terminal kinase kinase 1) (JNKK) (SAPK/ERK kinase 1) (SEK1).	301 ATGRFF P PKWNSVFD
74 DUS1_HUMAN	Dual specificity protein phosphatase 1 (EC 3.1.3.48) (EC 3.13.16) (MAP kinase phosphatase-1) (MKP-1) (Protein-tyrosine phosphatase CL100) (Dual specificity protein phosphatase hVH1).	335 TTTVF N FPV S IPVHS
75 DUS4_HUMAN	Dual specificity protein phosphatase 4 (EC 3.1.3.48) (EC 3.13.16) (Mitogen-activated protein kinase phosphatase-2) (MAP kinase phosphatase-2) (MKP-2) (Dual specificity protein phosphatase hVH2).	362 SQFVF S FPV S VG H S
76 EDA_HUMAN	Ectodysplasin A (Ectodermal dysplasia protein) (EDA protein).	134 ALLN E FP D E K PYSE

77 NPP2_HUMAN	78 EF1G_HUMAN	79 GAT2_HUMAN	80 CASL_HUMAN	81 ESR1_MOUSE	82 ETV2_HUMAN	83 ELK3_HUMAN	84 ELK4_HUMAN	85 ERF_HUMAN	86 ERF_HUMAN	87 ETV3_HUMAN	88 ETV3_HUMAN	89 IF33_HUMAN	90 IF37_HUMAN	91 FOL1_HUMAN	92 FXJ2_HUMAN	93 FXK1_MOUSE	94 FZD4_HUMAN	95 GCP6_HUMAN	96 GGPP_HUMAN	97 KG3A_HUMAN	98 GDF3_HUMAN	99 4206785	100 GBAS_HUMAN	101 HM21_HUMAN	102 4151328	103 HDA3_HUMAN	104 DBX1_MOUSE	105 HXCC_HUMAN	106 HMPH_HUMAN	107 HIK2_HUMAN
pyrophosphatase 2 (Phosphodiesterase I alpha) (PD-lalpha) (Autofaxin) [Includes: Alkaline phosphodiesterase I (EC 3.1.4.1); Nucleotide pyrophosphatase (EC 3.6.1.1); Elongation factor 1-gamma (EF-1-gamma) (eEF-1B gamma) (PRO1608); Endothelial transcription factor GATA-2].	Enhancer of filamentation 1 (HEF1) (CRK-associated substrate-related protein) (CAS-L) (CasL) (PP105) (Neural precursor cell expressed developmentally down-regulated 9).	Estrogen receptor (ER) (Estradiol receptor) (ER-alpha).	Ets translocation variant 2 (Ets-related protein 71).	ETS-domain protein ELK-3 (ETS-related protein NET) (ETS-related protein ERP) (SRF accessory protein 2) (SAP-2).	ETS-domain protein ELK-4 (Serum response factor accessory protein 1) (SAP-1).	ETS-domain transcription factor ERF (Ets2 repressor factor).	ETS-domain transcription factor ERF (Ets2 repressor factor).	ETS-related protein PE-1 (ETS translocation variant 3) (Fragment).	ETS-related protein PE-1 (ETS translocation variant 3) (Fragment).	Eukaryotic translation initiation factor 3 subunit 3 (eIF-3 gamma) (eIF3 p40 subunit) (eIF3h).	Eukaryotic translation initiation factor 3 subunit 7 (eIF-3 zeta) (eIF3 p66) (eIF3d).	Folate receptor alpha precursor (FR-alpha) (Folate receptor 1) (Folate receptor, adult) (Adult folate-binding protein) (FBP) (Ovarian tumor- associated antigen MOv18) (KB cells FBP).	Forkhead box protein J2 (Fork head homologous X).	Forkhead box protein K1 (Myocyte nuclear factor) (MNF).	Frizzled 4 precursor (Frizzled-4) (Fz-4) (hFz4) (FzE4).	Gamma-tubulin complex component 6 (GCP-6).	Geranylgeranyl pyrophosphate synthetase (GGPP synthetase) (GGPPSASE) (Geranylgeranyl diphosphate synthase) [Includes: Dimethylallyltransferase (EC 2.5.1.1); Geranyltransferase (EC 2.5.1.10); Farnesyltransferase (EC 2.5.1.29)].	Glycogen synthase kinase-3 alpha (EC 2.7.1.37) (GSK-3 alpha).	Growth/differentiation factor 3 precursor (GDF-3).	Guanine nucleotide-binding protein [Mus musculus]	High-mobility group protein 2-like 1 (HMGBCG protein).	High-risk human papilloma viruses E6 oncoproteins targeted protein E6TP1 alpha; putative GAP protein alpha [Homo sapiens].	Histone deacetylase 3 (HD3) (RPD3-2).	Homeobox protein DBX1.	Homeobox protein Hox-C12 (Hox-3F).	Homeobox protein PRH (Hematopoietically expressed homeobox) (Homeobox protein HEX).	Homeodomain-interacting protein kinase 2 (EC 2.7.1.-).			
676 MSYQGFLFPPYLSSSP	322 WYSEYRFPEELTQTF	167 GSHL_GFPPTPKEV	237 REKDYD DF PPPMRQAG	48 KPTVFN Y PEGAAYEF	25 AKLG FC FPDLALQGD	370 PSTLFQFQPTLNGHM	394 ANTLFQFQPSVLNSHG	138 GGSHFR FP SPTPSEV	5 ADTG FA FPDWAYKPE	168 ASSRFH FP LDTHSP	35 GGGGYQ FP DWAYKTE	78 ITNC FP FPQHTEDDA	331 GKERYN FP NPNPFPVE	176 QPFH FY FPTPTVLCN	404 NNTGEA FP PSDWCSNI	170 QQCTFR FP STAIKIQ	244 DSSRF SY PERPPIFL	1451 LPRA FA FPVDPQVQS	209 TEGKFS FP PTIHAWS	350 NYTEFK FP QIKAHPW	88 DQGFFLYPKKISQAS	104 NLPNF DF PPFEYEAH	136 NVPDF DF PPFEYEAH	207 DEESE Q QYPSQQATVK	1054 VSEYFKFPFRNNNKW	194 KYGNYFFPGTGD MYE	133 PP KT FAFPYEGFSQ	20 TGDTFYFPNFRASGA	97 GPLY PF PRTVNDYT	1067 AQAPYS FP HNSPSHG

108	IKAP_HUMAN	IkappaB kinase complex-associated protein (IKK complex-associated protein) (p150). Inositol-trisphosphate 3-kinase B (EC 2.7.1.127) (Inositol 1,4,5-trisphosphate 3-kinase) (IP3-3-kinase) (IP3K-B).	599	FPVRFPPYPCQTQTEL
109	IP3L_HUMAN		52	RGASFLFPPAESLSP
110	ITA5_HUMAN	Integrin alpha-5 precursor (Fibronectin receptor alpha subunit) (Integrin alpha-F) (VLA-5) (CD49e). Interstitial collagenase precursor (EC 3.4.24.7) (Matrix metalloproteinase-1) (MMP-1) (Fibroblast collagenase).	422	QGVVFVFPGGPGGLG
111	MM001_HUMAN		364	YSSEGEFPRTVKHID
112	13383464	kinesin-related protein HASH [Mus musculus]	46	NTRDFEMFPGPQNQMSG
113	KLF4_MOUSE	Kruppel-like factor 4 (Gut enriched kruppel-like factor) (Epithelial zinc-finger protein EZF).	134	STCSFSYPIRAGGDP
114	LAF4_HUMAN	LAF-4 protein (Lymphoid nuclear protein related to AF4).	325	EPTKEFPPNKDSQLV
115	LAMA_HUMAN	Lamin A/C (70 kDa laminin).	477	PLLTYRFPKFTLKA
116	4324434	large tumor suppressor 1 [Homo sapiens]	334	SSSKFNFNPSPGRPGMQ
117	930341	LAR-interacting protein 1a [Homo sapiens]	530	SVPDFRFPMADGHTD
118	EDG2_HUMAN	Lysophosphatidic acid receptor Edg-2 (LPA receptor 1) (LPA-1).	76	VNRRFHFPYIYLMAN
119	EDG7_HUMAN	Lysophosphatidic acid receptor Edg-7 (LPA receptor 3) (LPA-3).	57	KNRKFHFPPFYYLLAN
120	MM12_HUMAN	Macrophage metalloelastase precursor (EC 3.4.24.65) (HME) (Matrix metalloproteinase-12) (MMP-12) (Macrophage elastase) (ME).	367	SIHSEGFPNFVKKID
121	MKK2_HUMAN	MAP kinase-activated protein kinase 2 (EC 2.7.1.-) (MAPK-activated protein kinase 2) (MAPKAP kinase 2) (MAPKAPK-2).	280	RMGQYEFPNPEWSEV
122	MAPE_HUMAN	Melanoma antigen preferentially expressed in tumors (Preferentially expressed antigen of melanoma) (OPA-interacting protein 4) (OIP4).	140	RASLYSFPEPEAAQP
123	MAG3_HUMAN	Melanoma-associated antigen 3 (MAGE-3 antigen) (Antigen MZ2-D).	141	GNWQYFFFVFSKAS
124	TIM3_HUMAN	Metalloproteinase inhibitor 3 precursor (TIMP-3) (Tissue inhibitor of metalloproteinases-3) (MIG-5 protein).	168	MLSNFGYPGYQSCHKY
125	AMP1_HUMAN	Methionine aminopeptidase 1 (EC 3.4.11.18) (MetAP 1) (Peptidase M 1) (Fragment).	191	PLNYNNFPKSCTSV
126	MAPA_HUMAN	Microtubule-associated protein 1A (MAP 1A) (Proliferation-related protein p80) [Contains: MAP1 light chain LC2].	37	KPCCYIIPGGGRGDSA
127	RT29_HUMAN	Mitochondrial 28S ribosomal protein S29 (S29mt) (MRP-S29) (Death- associated protein 3) (DAP-3) (Ionizing radiation resistance conferring protein).	113	KNTSFAYPAIRYLLY
128	3133291	mitogen activated protein kinase [Homo sapiens]	259	MTGSFEEFPEEEWSQI
129	MK06_HUMAN	Mitogen-activated protein kinase 6 (EC 2.7.1.-) (Extracellular signal- regulated kinase 3) (ERK-3) (MAP kinase isoform p97) (p97-MAPK).	315	YMSIYSFPMDEPISS
130	M3K6_HUMAN	Mitogen-activated protein kinase kinase kinase 6 (EC 2.7.1.-).	800	HRPLFAFPDAVKQIL
131	TAB1_HUMAN	Mitogen-activated protein kinase kinase kinase 7 interacting protein 1 (TAK1-binding protein 1).	361	LVRNFGYPLGEMSQP
132	M4K2_HUMAN	Mitogen-activated protein kinase kinase kinase kinase kinase 2 (EC 2.7.1.37) (MAPK/ERK kinase kinase kinase 2) (MEK kinase kinase 2) (MEKK2) (Germinal center kinase) (GC kinase) (Rab8 interacting protein) (B lymphocyte serine/threonine protein kinase).	737	PELTEDFPIETVCL
133	MDR1_HUMAN	Multidrug resistance protein 1 (P-glycoprotein 1) (CD243 antigen).	1038	GEVVFNYPTRDIPV
134	MDR1_HUMAN	Multidrug resistance protein 1 (P-glycoprotein 1) (CD243 antigen).	395	RNVHFSYPSRKEVK1

135 MDR3_HUMAN	Multidrug resistance protein 3 (P-glycoprotein 3).	88 TAGNESFPVNFSL
136 MDR3_HUMAN	Multidrug resistance protein 3 (P-glycoprotein 3).	397 NDVHFSYPSRANVKI
137 DRNL_HUMAN	Muscle-specific DNase I-like precursor (EC 3.1.21.-) (DNase X) (XI).	242 TAAAFDFPFTSQLTE
138 6959304	MYB-binding protein 1A [Homo sapiens]	497 TKHPSFSPLENQARE
139 2706549	myc-intron-binding protein-1 [Mus musculus]	135 SEDLEPFPMPMHGSGG
140 MY15_HUMAN	Myosin XV (Unconventional myosin-15).	751 RGAAAFGFPGASPRAS
141 MM08_HUMAN	Neutrophil collagenase precursor (EC 3.4.24.34) (Matrix metalloproteinase-8) (MMP-8) (PMNL collagenase) (PMNL-CL).	364 DISNYGFPSSVQAID
142 KBF1_HUMAN	Nuclear factor NF-kappa-B p105 subunit (DNA-binding factor KBF1) (EBP-1) [Contains: Nuclear factor NF-kappa-B p50 subunit].	405 SFPHYGFP TYGGITF
143 KBF1_HUMAN	Nuclear factor NF-kappa-B p105 subunit (DNA-binding factor KBF1) (EBP-1) [Contains: Nuclear factor NF-kappa-B p50 subunit].	400 TGPGYSFPHYGFP TY
144 RI14_HUMAN	Nuclear factor RIP140 (Nuclear receptor interacting protein 1).	996 DNRTFSYPSGVVKTPV
145 RI14_HUMAN	Nuclear factor RIP140 (Nuclear receptor interacting protein 1).	905 NDLEFKYPAGHGSAS
146 N153_HUMAN	Nuclear pore complex protein Nup153 (Nucleoporin Nup153) (153 kDa nucleoporin).	421 RESGFSYPSVNFSLPA
147 NCR1_HUMAN	Nuclear receptor co-repressor 1 (N-Cor1) (N-Cor).	23 HSVQYTFPNTFRHQQE
148 RORG_HUMAN	Nuclear receptor ROR-gamma (Nuclear receptor RZR-gamma).	540 PPSPFSSFPMPNGGWS
149 DD21_HUMAN	Nucleolar RNA helicase II (Nucleolar RNA helicase Gu) (RH II/Gu) (DEAD-box protein 21).	136 RGVTFLFPIQAKTFH
150 NDK5_HUMAN	Nucleoside diphosphate kinase homolog 5 (NDK-H 5) (NDP kinase homolog 5) (nm23-H5) (Testis-specific nm23 homolog) (Inhibitor of p53-induced apoptosis-beta) (IP1A-beta).	137 REIRFMFPEVIVEPI
151 OXE2_HUMAN	Olfactory receptor 51E2 (Prostate specific G-protein coupled receptor) (HPRA).	150 RGSLFFFPLPLLIKR
152 ORC4_HUMAN	Origin-recognition complex subunit 4.	217 LMNSFGFPQVVKIFK
153 ORC5_HUMAN	Origin recognition complex subunit 5.	24 ERHHFSFSPSIFIFYGH
154 2645205	p160 myo-binding protein [Mus musculus]	495 TKQHFSFPLDDRNRG
155 PAXL_HUMAN	Paxillin.	114 EEHVYSFPNPKQKSAE
156 CYP6_HUMAN	Peptidyl-prolyl cis-trans isomerase like 2 (EC 5.2.1.8) (PPIase) (Rotamase) (Cyclophilin-60) (Cyclophilin-like protein Cyp-60).	45 SLQPFVYPVCTPDGI
157 CSKP_HUMAN	Peripheral plasma membrane protein CASK (EC 2.7.1.-) (hCASK) (Calcium/calmodulin-dependent serine protein kinase) (Lin-2 homolog).	763 HPDRFAYPIPHTRP
158 PECL_HUMAN	Peroxisomal 3,2-trans-enoyl-CoA isomerase (EC 5.3.3.8) (Dodecenoyl-CoA delta-isomerase) (D3,D2-enoyl-CoA isomerase) (DBI-related protein 1) (DRS-1) (Hepatocellular carcinoma-associated antigen 88).	246 GCSSYTFPKIMSPAK
159 PPAR_HUMAN	Peroxisome proliferator activated receptor alpha (PPAR-alpha).	417 PDDIELFPKLLQKMA
160 PPAS_MOUSE	Peroxisome proliferator activated receptor delta (PPAR-delta) (PPAR-beta) (Nuclear hormone receptor 1) (NUC1).	389 PDSQYLFPKLLQKMA
161 P85A_MOUSE	Phosphatidylinositol 3-kinase regulatory alpha subunit (PI3-kinase p85-alpha subunit) (PtIns-3-kinase p85-alpha) (PI3K).	269 SPVLFRFPAASSDNT
162 PI4K_HUMAN	Phosphatidylinositol 4-kinase alpha (EC 2.7.1.67) (PI4-kinase) (PtIns-4-kinase) (PI4K-alpha).	628 GLDLFVFPYRVVATA
163 PTEN_HUMAN	Phosphatidylinositol-3,4,5-trisphosphate 3-phosphatase PTEN (EC 3.1.3.67) (Mutated in multiple advanced cancers 1).	237 KFMYFEFPQPLPVCG

164	P11B_HUMAN	Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit, beta isoform (EC 2.7.1.153) (PI3-kinase p110 subunit beta) [PtdIns-3-kinase p110] (PI3K) (PI3Kbeta).	608	ELLD F NYPDQYVREY
165	P11D_HUMAN	Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit, delta isoform (EC 2.7.1.153) (PI3-kinase p110 subunit delta) [PtdIns- 3-kinase p110] (PI3K) (p110delta).	581	ELLD F SFPDCHVGSF
166	P11D_HUMAN	Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit, delta isoform (EC 2.7.1.153) (PI3-kinase p110 subunit delta) [PtdIns- 3-kinase p110] (PI3K) (p110delta).	161	AWLQYSFPLQLEPSA
167	940231	phosphodiesterase A' subunit [Homo sapiens]	366	ADEYETTFPKGPVDET
168	22758919	phosphoinositide 3 kinase P110delta [Mus musculus]	580	ELLD F SFPDCYVGSF
169	2696236	phospholipase B [Rattus norvegicus]	42	TLKNF S FPCKPKKLE
170	PLEK_HUMAN	Pleckstrin (Platelet p47 protein).	213	PDAFY Y FPDSGFFCE
171	3002588	Plenty of SH3s; POSH [Mus musculus]	376	TGPAFTFPDSDVYQA
172	PAB5_HUMAN	Polyadenylate-binding protein 5 (Poly(A)-binding protein 5) (PABP 5).	176	YVGRFKFPEERAAEV
173	PAB5_HUMAN	Polyadenylate-binding protein 5 (Poly(A)-binding protein 5) (PABP 5).	62	GYVNFRFPADAEWAL
174	15667468	polyploidy associated protein kinase PAPK-A [Mus musculus]	402	SELE Q FPDDKDWPVW
175	DP1_HUMAN	Polyposis locus protein 1 (TB2 protein).	56	NLIG F GYPAYISIKA
176	CIQ3_HUMAN	Potassium voltage-gated channel subfamily KQT member 3 (Potassium channel KQT-like 3).	587	KGSAFTFPSSQSQSPRN
177	MV10_HUMAN	Potassium voltage-gated channel subfamily MOV-10.	601	KKGEY Y VEPAKKLQE
178	A11A_HUMAN	Potential phospholipid-transporting ATPase IS (EC 3.6.3.1) (Fragment).	552	KNYCF I FPQFLYQFFF
179	4097902	potential transcriptional repressor Not4hp [Mus musculus]	485	VYN S FGFPQQAARYP
180	PRD2_HUMAN	PR-domain zinc finger protein 2 (Retinoblastoma protein-interacting zinc-finger protein) (IMTE-binding protein) (MTB-ZF).	354	VFET E MFPCQHQHCRK
181	DDX6_HUMAN	Probable ATP-dependent RNA helicase p54 (Oncogene RCK) (DEAD-box protein 6).	405	VVINF E DFPKLAETYL
182	GPR4_HUMAN	Probable G protein-coupled receptor GPR4 (GPR19).	186	VVF G ELFPWALMLLS
183	GP68_HUMAN	Probable G protein-coupled receptor GPR68 (Ovarian cancer G protein- coupled receptor 1) (OGR-1).	190	FLVGELFPICLLLAS
184	K682_HUMAN	Probable RNA-binding protein KIAA0682.	446	AFIT E MFPEHAVKAY
185	MN1_HUMAN	Probable tumor suppressor protein MN1.	455	QE A R D EPGSAGVDR
186	FAFX_HUMAN	Probable ubiquitin carboxyl-terminal hydrolase FAF-X (EC 3.1.2.15) (Ubiquitin thiolesterase FAF-X) (Ubiquitin-specific processing protease FAF-X) (Deubiquitinating enzyme FAF-X) (Fat facets protein related, X-linked) (Ubiquitin-specific protease 9, X chro	1805	FNDY E FPRELDMEP
187	FAFY_HUMAN	Probable ubiquitin carboxyl-terminal hydrolase FAF-Y (EC 3.1.2.15) (Ubiquitin thiolesterase FAF-Y) (Fat facets protein related, Y-linked) (Ubiquitin-specific protease 9, Y chro	1616	RDDVFGYPHQQFEDKP
188	PDC2_HUMAN	Programmed cell death protein 2 (Zinc finger protein Rp-8).	183	PDHNF L FEFEVIE
189	38588885	proliferation potential-related protein [Mus musculus]	742	DTTLE V FPSREDATP
190	KPCM_HUMAN	Protein kinase C, mu type (EC 2.7.1.-) (nPKC-mu) (Protein kinase D).	794	QNAAE F MYPPNPWKEI
191	KPCN_HUMAN	Protein kinase C, nu type (EC 2.7.1.-) (nPKC-nu) (Protein kinase EPK2).	787	QNAAE F MYPPNPWREI
192	15080775	protein kinase NYD-SP5 [Homo sapiens]	97	DQKSE I FPQESEGTF
193	PP3D_HUMAN	Protein phosphatase 1, regulatory subunit 3D (Protein phosphatase 1, regulatory subunit 6) (Protein phosphatase 1 binding subunit R6).	234	DVFTE G FPVPPFLLE

194	10567793	protein tyrosine phosphatase BK [Mus musculus]	262	RDRREHFPEETTPETP
195	11440012	protein tyrosine phosphatase D30 [Rattus norvegicus]	261	RDRFHPEETTPETP
196	23268287	protein tyrosine phosphatase receptor-like protein J [Mus musculus]	172	TNNSEAFPESNETQA
		Protein tyrosine phosphatase, non-receptor type 13 (EC 3.1.3.48) (Protein-tyrosine phosphatase PTPL1) (Fas-associated protein- (PTP-E1) (hPTP-E1) (PTP-BAS) (Protein-tyrosine phosphatase PTPL1) (Fas-associated protein-tyrosine phosphatase 1) (FAP-1).		
197	PTND_HUMAN	Proteinase activated receptor 2 precursor (PAR-2) (Thrombin receptor-like 1) (Coagulation factor II receptor-like 1).	1704	ICTM YY PQKIPNKP
198	PAR2_HUMAN	Protein-tyrosine phosphatase eta precursor (EC 3.1.3.48) (R-PTP-eta) (HPTP beta-like tyrosine phosphatase).	172	TNNT FA FPESNETQA
199	PTPJ_MOUSE	Protein-tyrosine phosphatase zeta precursor (EC 3.1.3.48) (R-PTP- zeta) (Phosphacan) (3F8)	247	AIGVEL FPA FLTASA
200	PTPZ_RAT	Protein-tyrosine phosphatase zeta precursor (3H1 keratan sulfate proteoglycan) (chondroitin sulfate proteoglycan) (3F8)	1565	TSTDF ES PDVNNEKDA
201	PTPZ_RAT	Protein-tyrosine phosphatase zeta precursor (EC 3.1.3.48) (R-PTP- zeta) (Phosphacan) (3F8)	720	HYSTFAY PP TEVTSH
202	FAT2_HUMAN	Protein-tyrosine phosphatase zeta precursor (EC 3.1.3.48) (R-PTP- zeta) (Phosphacan) (3F8)	4222	LYGGFP PP LEMENKR
203		chondroitin sulfate proteoglycan) (3H1 keratan sulfate proteoglycan), Protocadherin Fat 2 precursor (hFat2) (Multiple epidermal growth factor-like domains 1).	100	NNQLFRFPATSPLKT
204	GP40_HUMAN	Putative cell cycle control protein [Homo sapiens]	244	NVASFLY PNL GGSWR
205	RBM9_HUMAN	Putative RNA-binding protein 9 (RNA binding motif protein 9).	251	IIPGFPYPTAATTAA
206	6007826	rab escort protein-2 [Mus musculus]	370	GNTP FI FPLYGHGEI
207	RAE1_HUMAN	Rab proteins geranylgeranyltransferase component A 1 (Rab escort protein 1) (REP-1) (Chorioideraemia protein) (TCD protein).	369	GNTP FL FPLYGQGEL
208	RAE2_HUMAN	Rab proteins geranylgeranyltransferase component A 2 (Rab escort protein 2) (REP-2) (Chorioideraemia-like protein).	371	GNTP FL FPLYGQGEI
209	RBPL_HUMAN	Recombining binding protein suppressor of hairless-like protein (Transcription factor RBP-L).	311	HKCAEFQ FP PGSPPGGG
210	RB_HUMAN	Retinoblastoma-associated protein (PP110) (P105-RB) (RB).	786	PRSPYK FP SSPLRIP
211	RBB5_HUMAN	Retinoblastoma-binding protein 5 (RBBP-5) (Retinoblastoma-binding protein RBQ-3).	100	CDQRFR FP SPILKVQ
212	RBB8_HUMAN	Retinoblastoma-binding protein 8 (RBBP-8) (CBP interacting protein) (CtIP) (Retinoblastoma-interacting protein and myosin-like) (RIM).	467	KENAF FP PMNDNQFSM
213	RBL2_HUMAN	Retinoblastoma-like protein 2 (130 kDa retinoblastoma-associated protein) (PRB2) (P130) (RBR-2).	170	FQDIEK Y PQEQQPRA
214	12053793	retinoid-acid induced protein 1 [Homo sapiens]	1830	PISL FS FP PL PQQF
		Rho guanine nucleotide exchange factor 2 (GEF-H1 protein) (Proliferating cell nucleolar antigen p40).	505	KDQKYI FT LDKPSV
215	ARH2_HUMAN	Rho-GTPase-activating protein 7 (Rho-type GTPase-activating protein 7) (Deleted in liver cancer 1 protein) (Dlc-1) (HP protein) (StAR-related lipid transfer protein 12) (StARD12) (START domain-containing protein 12).	34	LYEDFLFP IDIS LVK
216	RHG7_HUMAN	RNA-binding protein with multiple splicing (RBP-MS).	163	PPPAFT TY PASLHAQM
217	RBMS_HUMAN	Runt-related transcription factor 2 (Core-binding factor, alpha 1 subunit) (CBF-alpha 1) (Acute myeloid leukemia 3 protein) (Oncogene AML-3) (Polyomavirus enhancer binding protein 2 alpha A subunit) (PEBP2-alpha A) (PEA2-alpha A) (SL3-3 enhancer factor 1	450	SSGSSYQ FP PMVPGGDR

219 SEN2_HUMAN	66 AASLEGFPFQLTTKP
220 SEN2_HUMAN	437 VFSTEFYPKLKSGGY
221 SEP1_HUMAN	195 EHIVQFPECDSDED
222 SEP4_HUMAN	Septin 1 (LARP) (Serologically defined breast cancer antigen NY-BR-24) (EC 3.4.22.-) (Sentrin/SUMO-specific protease SENP2).
223 SEP5_HUMAN	Septin 4 (Peanut-like protein 2) (Brain protein H5) (Cell division control-related protein 2) (hCDCREL-2) (hCDCREL-1).
224 SEP7_HUMAN	Septin 5 (Peanut-like protein 1) (Cell division control related protein 1) (CDCREL-1).
225 STK6_HUMAN	Septin 7 (CDC10 protein homolog).
226 STKD_MOUSE	Serine/threonine kinase 6 (EC 2.7.1.37) (Serine/threonine kinase 15) (Aurora/IPL1-related kinase 1) (Aurora-related kinase 1) (hARK1) (Aurora-A) (Breast-tumor-amplified kinase).
227 KPT3_HUMAN	Serine/threonine protein kinase 13 (EC 2.7.1.37) (Aurora/p11Eg2 protein 1).
228 2ACA_HUMAN	Serine/threonine protein kinase 19 (EC 2.7.1.37) (RP1 protein) (G11 protein).
229 ST19_HUMAN	Serine/threonine-protein kinase MAK (EC 2.7.1.-) (Male germ cell- associated kinase) (Protein kinase RCK).
230 MAK_MOUSE	Serine/threonine-protein kinase MAK (EC 2.7.1.-) (Male germ cell- associated kinase).
231 MAK_HUMAN	Serine/threonine-protein kinase PCTAIRE-2 (EC 2.7.1.-).
232 KPT2_HUMAN	Serine/threonine-protein kinase ULK1 (EC 2.7.1.-) (Unc-51-like kinase 1).
233 ULK1_HUMAN	Serine/threonine-protein kinase and activator of transcription 5A.
234 ST5A_HUMAN	Signal transducer and activator of transcription 5B.
235 ST5B_HUMAN	Signal transduction protein CBL (Proto-oncogene c-CBL).
236 CBL_HUMAN	Signal transduction protein CBL-B (SH3-binding protein CBL-B).
237 CBLB_HUMAN	Signal transduction protein CBLB (SH3-binding protein CBL-B).
238 6649242	Splicing coactivator subunit SRM300 [Homo sapiens] (Pre-mRNA splicing factor SF3B subunit 3 (Spliceosome associated protein 130) (SAP 130) (SF3b130) (Pre-mRNA splicing factor SF3B 130 kDa subunit).
239 S3B3_HUMAN	Stac protein (SRC homology 3 and cysteine-rich domain protein).
240 STAC_HUMAN	T-box transcription factor TBX18 (T-box protein 18) (Fragment).
241 TX18_HUMAN	T-box transcription factor TBX20 (T-box protein 20) (Fragment).
242 TX20_HUMAN	T-box transcription factor TBX3 (T-box protein 3).
243 TBX3_HUMAN	T-box transcription factor TBX6 (T-box protein 6).
244 TBX6_HUMAN	TFIIB-alpha and beta like factor (ALF).
245 T2AY_HUMAN	TFIIB basal transcription factor complex p34 subunit (Basic transcription factor 2 34 kDa subunit)
246 TFH3_HUMAN	(BTF2-p34) (General transcription factor IIH polypeptide 3).
247 TOB1_HUMAN	Tob1 protein (Transducer of erbB-2 1).
248 GAT3_HUMAN	Trans-acting T-cell specific transcription factor GATA-3.
249 GAT4_HUMAN	Transcription factor GATA-4 (GATA binding factor-4).
250 GAT5_HUMAN	Transcription factor GATA-5 (GATA binding factor-5).
251 TF65_MOUSE	Transcription factor p65 (Nuclear factor NF-kappa-B p65 subunit).

252	TCL5_HUMAN	Transcription factor-like 5 protein (Cha transcription factor) (HPV-16 E2 binding protein 1) (E2BP-1).	203	TALQFTYPLFTTNAC
253	TF1A_HUMAN	Transcription intermediary factor 1-alpha (TIF1-alpha) (Tripartite motif protein 24).	729	PPENYDFPVVVKQE
254	ERG_MOUSE	Transcriptional regulator ERG (Fragment).	52	GGAAFIFPNTSVYPE
255	TERA_HUMAN	Transitional endoplasmic reticulum ATPase (TER ATPase) (15S Mg(2+)-ATPase p97 subunit)	767	GFGSFRFPSGNQGGA
256	E2BE_HUMAN	(Valosin containing protein) (VCP) [Contains: Valosin]. Translation initiation factor eIF-2B epsilon subunit (eIF-2B GDP-GTP exchange factor).	220	GLRRFAFPLSLFQGS
257	SSRA_HUMAN	Transloco-associated protein, alpha subunit precursor (TRAP-alpha) (Signal sequence receptor alpha subunit) (SSR-alpha).	120	LDASSEFRYPQDYQFYI
258	TRIO_HUMAN	Triple functional domain protein (PTPRF interacting protein).	2437	RPGSE T PGDSDLQ
259	1113923	tyrosine phosphatase-like protein IA-2a [Rattus norvegicus]	947	SKDKF E PLTPYGE
		Tyrosine-protein kinase BTK (EC 2.7.1.112) (Bruton's tyrosine kinase) (Agammaglobulinaemia		
		Tyrosine-protein kinase (ATK) (B cell progenitor kinase) (BPK).	94	IIERF P YPFQWVYDE
		Tyrosine-protein kinase JAK2 (EC 2.7.1.112) (Janus kinase 2) (JAK-2).	114	YRIRFY F PRWYCSCGS
		Tyrosine-protein kinase JAK3 (EC 2.7.1.112) (Janus kinase 3) (JAK-3) (Leukocyte Janus kinase) (L-JAK).	100	YRIRFY F PNWFGLEK
		Tyrosine-protein kinase SYK (EC 2.7.1.112) (Spleen tyrosine kinase).	292	RIKS S EPKPGHRKs
		Tyrosine-protein kinase transmembrane receptor ROR1 precursor (EC 2.7.1.12) (Neurotrophic tyrosine kinase, receptor-related 1).	785	RYPN Y MFPSQGITPQ
		Tyrosine-protein kinase transmembrane receptor ROR2 precursor (EC 2.7.1.12) (Neurotrophic tyrosine kinase, receptor-related 2).	230	SFCH E V F PLCDARSR
		Ubiquitin carboxyl-terminal hydrolase 26 (EC 3.1.2.15) (Ubiquitin thiolesterase 26) (Ubiquitin-specific processing protease 26) (Deubiquitinating enzyme 26).	223	SKPG E GF F ETNYPE
		Ubiquitin carboxyl-terminal hydrolase 8 (EC 3.1.2.15) (Ubiquitin thiolesterase 8) (Ubiquitin-specific processing protease 8) (Deubiquitinating enzyme 8).	330	ISLDFT Y PSLEESIP
		Ubiquitin conjugation factor E4 B (Ubiquitin-fusion degradation protein 2).	537	DSDYFKYPLMALGEL
		Ubiquitin ligase E3 alpha-II [Homo sapiens]	80	EDPA E GF P KLEQANK
		Ubiquitin--protein ligase EDD (EC 6.3.2.-) (Hyperplastic discs protein homolog) (hHYD) (Progesterin induced protein).	274	DISYFG Y GPSFRRSSL
		Valyl-tRNA synthetase 2 (EC 6.1.1.9) (Valine-tRNA ligase 2) (ValRS 2) (G7a).	1001	GFOAY D FPAVTTAQY
		Vault poly(ADP-ribose) polymerase (EC 2.4.2.30) (VPARP) (193-kDa vault protein) (PARP-related/lipid-rich H5/proline-rich) (PH5P).	921	YKELF S YPKHTTSNT
		Vinexin (SH3-containing adaptor molecule-1) (SCAM-1).	565	RRTG E FSFPTQEPRPQ
		Vinexin (SH3-containing adaptor molecule-1) (SCAM-1).	352	GRRD F V P SSSTRDPS
		Werner syndrome helicase.	577	KSLC F QY P PVYVGK1
		Wiskott-Aldrich syndrome protein family member 2 (WASP-family protein member 2) (Verprolin homology domain-containing protein 2).	270	PAAEF S YPVDNQRGS
		Wiskott-Aldrich syndrome protein family member 2 (WASP-family protein member 2) (Verprolin homology domain-containing protein 2).	218	KLGP E GY P P T LVYQN
		Wiskott-Aldrich syndrome protein family member 2 (WASP-family protein member 2) (Verprolin homology domain-containing protein 2).	149	PSGAY V F P PPVANGM
276	WAS2_HUMAN	WW domain binding protein 2 (WBP-2).		
277	WAS2_MOUSE			
278	WBP2_MOUSE			

279 **ZN44**_HUMAN Zinc finger protein 44 (Zinc finger protein K0X7) (Gonadotropin inducible transcription repressor-2) (GIOT-2).
 280 **HRX**_HUMAN Zinc finger protein HRX (ALL-1) (Trithorax-like protein).
 281 **Z287**_HUMAN Zinc finger protein ZNF287.

TABLE 3: CARDIOVASCULAR

Accession Code	Target Description	Amino Acid	Target Sequence
1 ACHB _HUMAN	Acetylcholine receptor protein, beta chain precursor.	395	PPSDFLFPKPKNRFQP
2 CYA4 _HUMAN	Adenylate cyclase, type IV (EC 4.6.1.1) (ATP pyrophosphate-lyase) (Adenylyl cyclase).	679	ITSLEFFPTSSDCPF
3 GAT2 _HUMAN	Endothelial transcription factor GATA-2.	167	GSHLFGFPPTPPKEV
4 CXA1 _HUMAN	Gap junction alpha-1 protein (Connexin 43) (Cx43) (Gap junction 43 kDa heart protein). Guanine nucleotide-binding protein G(S), alpha subunit (Adenylyl cyclase-stimulating G alpha protein).	330	HAQPFDEPDDNNQNSK
5 GBAS _HUMAN	I kappa B kinase complex-associated protein (IKK complex-associated protein) (p150).	136	NVPDFDFPPEFYEHA
6 IKAP _HUMAN	Leucine-rich repeat-containing G protein-coupled receptor 6.	599	FPVRFPYPCTQTEL
7 LGR6 _HUMAN	Leucine-rich skeletal muscle, extraocular (MyHC-eo).	751	GLETYGFPSVTLISC
8 MYHD _HUMAN	Myosin heavy chain, skeletal muscle, extraocular (MyHC-eo). NDRG4 protein (Brain development-related molecule 1) (Vascular smooth muscle cell associated protein-8) (SMAP-8).	306	STNPFDFPFVSSQGEV
9 NDR4 _HUMAN	Neuronal acetylcholine receptor protein, beta-3 chain precursor.	80	FPQGQYQFPSMSMELQAA
10 ACHO _RAT	P2X purinoreceptor 1 (ATP receptor) (P2X1) (Purinergic receptor).	358	PMDRFSFPDPDGKESDT
11 P2X1 _HUMAN	P2X purinoreceptor 7 (ATP receptor) (P2X7) (Purinergic receptor) (P2Z receptor).	86	DVADYYVFPAPAQQDNSF
12 P2X7 _HUMAN	Probable G protein-coupled receptor GPR4 (GPR19).	89	DTADYYTFPLQGQNSFF
13 GPR4 _HUMAN	Probable G protein-coupled receptor GPR68 (Ovarian cancer G protein-coupled receptor 1) (OGR-1).	186	VFGVFLFPWALMLLS
14 GP68 _HUMAN	Probable P2Y purinoreceptor GPR17 (P2Y-like receptor) (R12).	190	FLVGFLLFPICLLLAS
15 GP17 _HUMAN	Putative G protein-coupled receptor GPR40.	225	LAVAFTFPFITTVTC
16 GP40 _HUMAN	Putative P2Y purinoreceptor FKSG79.	244	NVASELYPNLGGSWR
17 FK79 _HUMAN	Relaxin receptor 1 (Leucine-rich repeat-containing G protein-coupled receptor 7).	244	APYHFSFPPLDFFLVKS
18 LGR7 _HUMAN	Type-1 angiotensin II receptor (AT1) (AT1AR).	734	KPDLFTYPCEMSLIS
19 AG2R _HUMAN	Vascular non-inflammatory molecule 2 precursor (Vanin 2) (Glycosylinositol-phosphatidylinositol-anchored protein GPI-80) (FOAP-4 protein).	200	NILGFLFPFLILTS
20 VNN2 _HUMAN	Very large G protein-coupled receptor 1 [Mus musculus].	435	FGTEYYFPEVILLTEI
21 16904210	Von Willebrand factor precursor (vWF).	1971	PYGVFFIFPNKTRPLS
22 VWF _HUMAN	Williams-Beuren syndrome chromosome region 14 protein (WS basic-helix-loop-helix zipper protein) (WS-bHLH) (Mlx interactor).	879	DGLKYLFPGECCQYVL
23 WS14 _HUMAN		431	FSPRFPFPPTVPPAPG

TABLE 4: INFLAMMATION

Accession Code	Target Description	Amino Acid	Target Sequence
1 A1M2_HUMAN	Adaptor-related protein complex 1, mu 2 subunit (Mu-adaptin 2) (Adaptor protein complex AP-1 mu-2 subunit) (Golgi adaptor HA1/AP1 adaptin mu-2 subunit) (Clathrin assembly protein assembly protein complex 1 medium chain 2) (AP-mu chain family member mu1B).	116 ELMDEGFPQTTDSKI	
2 AIF1_HUMAN	Allergen inflammatory factor-1 (AIF-1) (Ionized calcium-binding adapter molecule 1) (G1).	97 SGETFSYPPDFFLRRMML	
3 APL3_HUMAN	Apolipoprotein L3 (Apolipoprotein L-III) (ApoL-III) (TNF-inducible protein CG12-1) (CG12_1).	23 QVVTFTFFGFFQGQIS	
4 CAB1_HUMAN	Calcineurin-binding protein Cabin 1 (Calcineurin inhibitor) (CAIN).	947 FYCLYSFPSKSKSKAR	
5 CEBA_HUMAN	CCAAT/enhancer binding protein epsilon (C/EBP alpha).	102 GGGDFDYPGAPAGPG	
6 CEBE_RAT	CCAAT/enhancer binding protein epsilon (C/EBP epsilon) (C/EBP-related protein 1).	84 DPRPFAYPSHTFGPD	
7 CO4_HUMAN	Complement C4 precursor [Contains: C4A anaphylatoxin].	915 ARGGSFEEFPYGDAVSK	
8 CCR3_HUMAN	C-X-C chemokine receptor type 3 (CXCR-R3) (CXCR-3) (CD183 antigen).	201 THCQYNNFPQVQVGRTEL	
9 GAT2_HUMAN	Endothelial transcription factor GATA-2.	167 GSHLFGFPPTPPKEV	
10 FYB_HUMAN	FYN-binding protein (FYB-120/130) (p120/p130) (SLP-76 associated phosphoprotein) (SLAP-130).	125 SKPTFPWPPGNGKPSL	
11 28274770	HMG-box protein SOX21 [Mus musculus]	88 KKDKKFAFPVPPYGLGS	
12 IKAP_HUMAN	IkappaB kinase complex-associated protein (IKK complex-associated protein) (p150). Interferon regulatory factor 4 (IRF-4) (Lymphocyte specific interferon regulatory factor) (LSIRF) (NF-EM5) (Multiple myeloma oncogene 1).	599 FPVRFPYPPCTQTELA	
13 IRF4_HUMAN	Interleukin enhancer-binding factor 1 (Cellular transcription factor ILF-1).	288 DQVLFYPYPEDNGQRK	
14 ILF1_HUMAN	Interleukin enhancer-binding factor 1 (Cellular transcription factor ILF-1).	134 RVCTTERFPSTNIKIT	
15 IRA2_HUMAN	Interleukin-1 receptor-associated kinase-2 (EC 2.7.1.-) (IRAK-2).	280 QFH5FIYPPYMANGSL	
16 BAT2_HUMAN	Large proline-rich protein BAT2 (HLA-B-associated transcript 2) (G2).	249 MMPPEMYPYLPFPPP	
17 LRBA_HUMAN	Large proline-rich protein BAT2 (HLA-B-associated transcript 2) (G2). Lipopolysaccharide-responsive and beige-like anchor protein (CDC4-like protein) (Beige-like protein).	203 PDAFFENFPGKSAAAI	
18 MM12_HUMAN	Macrophage metalloelastase precursor (EC 3.4.24.65) (HME) (Matrix metalloproteinase-12) (MMP-12) (Macrophage elastase) (ME).	367 SIHSEGFEPNFKKID	
19 NDR4_HUMAN	NDRG4 protein (Brain development-related molecule 1) (Vascular smooth muscle cell associated protein-8) (SMAP-8).	80 FPQGYQFQPSMEQQLAA	
20 NOCT_HUMAN	Nocturnin (CCR4 protein homolog).	404 RLPSENYPPSDHLSLV	
21 KBF1_HUMAN	Nuclear factor NF-kappa-B p105 subunit (DNA-binding factor KBF1) (EBP-1) [Contains: Nuclear factor NF-kappa-B p50 subunit].	405 SFPHYGFPTYGGITF	
22 KBF1_HUMAN	Nuclear factor NF-kappa-B p105 subunit (DNA-binding factor KBF1) (EBP-1) [Contains: Nuclear factor NF-kappa-B p50 subunit].	400 TGPGYSFPHYGFPTY	
23 PAR2_HUMAN	Proteinase activated receptor 2 precursor (PAR-2) (Thrombin receptor-like 1) (Coagulation factor II receptor-like 1).	247 AIGVELFFPAFLTASA	
24 LGR7_HUMAN	Relaxin receptor 1 (Leucine-rich repeat-containing G protein-coupled receptor 7).	734 KPDLLFTYPCEMSLIS	
25 RRA_HUMAN	Retinoic acid receptor alpha (RAR-alpha).	22 PPVAFFFPMLGGGLS	
26 ST5A_HUMAN	Signal transducer and activator of transcription 5A.	664 SYLIYVFPDRPKDEV	
27 ST5B_HUMAN	Signal transducer and activator of transcription 5B.	664 NYLIYVFPDRPKDEV	
28 SX12_HUMAN	SOX-12 protein (SOX-22 protein).	281 GTSHEFFPDYCTPEV	

29	15277232	TNFalpha-inducible ATP-binding protein [Homo sapiens]	570	YTVRFTFPDPPLSP
30	TLR1_HUMAN	Toll-like receptor 1 precursor (Toll/interleukin-1 receptor-like) (T1L).	308	VSDVGFPPQSYIEI
31	TLR4_HUMAN	Toll-like receptor 4 precursor (hToll).	71	SYSFSFPELQVLDL
32	GAT3_HUMAN	Trans-acting T-cell specific transcription factor GATA-3.	147	SPHL FTFP TPPKDV
33	TF65_MOUSE	Transcription factor p65 (Nuclear factor NF-kappa-B p65 subunit).	345	APQPYTFP ASL STIN
34	SX11_HUMAN	Transcription factor SOX-11.	407	LGSH FEFP DYCTPEL
35	SX21_HUMAN	Transcription factor SOX-21.	88	KKDKFAFPV Y GLGG
36	SOX4_HUMAN	Transcription factor SOX-4.	440	SGSH FEFP DYCTPEV
37	SOX6_HUMAN	Translational-associated protein, alpha subunit precursor (TRAP-alpha) (Signal sequence receptor alpha subunit) (SSR-alpha).	285	AQQGEL FPPG GI TY KP
38	SSRA_HUMAN	Tumor necrosis factor receptor superfamily member 11B precursor (Osteoprotegerin)	120	LDASF RY PQDYQFYI
39	T11B_HUMAN	(Osteoclastogenesis inhibitory factor).	351	HSKTYH FP KTVTQSL
		Tumor necrosis factor receptor superfamily member 13B (Transmembrane activator and CAML interactor).	228	ETCS FC EPECRAP TQ
40	T13X_HUMAN	Type-1 angiotensin II receptor (AT1) (AT1AR).	200	NIL FL FPFLIILTS
41	AG2R_HUMAN	Tyrosine-protein kinase JAK2 (EC 2.7.1.112) (Janus kinase 2) (JAK-2).	114	YRIRFY FP RWYC SGS
42	JAK2_HUMAN	Tyrosine-protein kinase JAK3 (EC 2.7.1.112) (Janus kinase 3) (JAK-3) (Leukocyte janus kinase) (L-JAK).	100	YRIRFY FP NW FG LEK
43	JAK3_HUMAN	Vascular non-inflammatory molecule 3 precursor (Vanin 3).	185	PEIQFDFPKDSELVT
44	VNN3_HUMAN	Vascular non-inflammatory molecule 3 precursor (Vanin 3).	436	FGTRYYFPQIILSGS
45	VNN3_HUMAN	Vinexin (SH3-containing adaptor molecule-1) (SCAM-1).	565	RRTGFSFPTQEPRPQ
46	VINE_HUMAN	Vinexin (SH3-containing adaptor molecule-1) (SCAM-1).	352	GRRDF VY PSSTRDPS
47	VINE_HUMAN	Zinc finger protein 40 (Human immunodeficiency virus type I enhancer- binding protein 1) (HIV-EP1) (Major histocompatibility complex binding protein 1) (MBP-1) (Positive regulatory domain II binding factor 1) (PRDII-BF1).	1255	KSEKF SW PQRSETLS
48	ZEP1_HUMAN			

TABLE 5: METABOLIC DISORDERS

Accession Code	Target Description	Amino Acid	Target Sequence
1 PIP3_HUMAN	1-phosphatidylinositol-4,5-bisphosphate phosphodiesterase beta 3 (EC 3.1.4.11) (Phosphoinositide phospholipase C) (PLC-beta-3).	777	DEEPDF FP KV ML P TL
2 AAK1_HUMAN	5'-AMP-activated protein kinase, catalytic alpha-1 chain (EC 2.7.1.-) (AMPK alpha-1 chain).	273	DL PK Y LF FP ED PSYSS
3 AAK2_HUMAN	5'-AMP-activated protein kinase, catalytic alpha-2 chain (EC 2.7.1.-) (AMPK alpha-2 chain).	271	DL PSY Y LF FP ED PSYDA
4 ACDV_HUMAN	Acy-CoA dehydrogenase, very-long-chain specific, mitochondrial precursor (EC 1.3.99.-) (VLCAD).	84	TDQVF FP PSV LN EEQ
5 ANDR_HUMAN	Androgen receptor (Dihydrotestosterone receptor).	359	SRDYY NF PL LA AGPP
6 ANDR_HUMAN	Androgen receptor (Dihydrotestosterone receptor).	547	LPIDYY FP QQ KT CL
7 APB_HUMAN	Apolipoprotein B-100 precursor (Apo B-100) [Contains: Apolipoprotein B-48 (Apo B-48)].	4198	NFPRF QEP GGKPGIY T

8 APL3_HUMAN	Apolipoprotein L3 (Apolipoprotein L-III) (Apol-III) (TNF-inducible protein CG12-1) (CG12-1).	23 QVVT T FPFGFQGIS
9 AQP1_HUMAN	Aquaporin-CHIP (Water channel protein for red blood cells and kidney proximal tubule) (Aquaporin 1) (AQP-1) (Urine water channel).	31 SALGF K YPVGNINQTA
10 ABC1_HUMAN	ATP-binding cassette, sub-family A, member 1 (ATP-binding cassette transporter 1) (ATP-binding cassette 1) (ABC-1) (Cholesterol efflux regulatory protein).	78 NN P C F RYPTPGEAPG
11 ABF2_HUMAN	ATP-binding cassette, sub-family F, member 2 (Iron inhibited ABC transporter 2) (HUSSY-18).	379 KTL S YFP P C G KIPP
12 HEXA_HUMAN	Beta-hexosaminidase alpha chain precursor (EC 3.2.1.52) (N-acetyl- beta-glucosaminidase) (Beta-N-acetylhexosaminidase) (Hexosaminidase A).	212 PYES F TEPELMRKGS
13 AB11_HUMAN	Bile salt export pump (ATP-binding cassette, sub-family B, member 11).	1081 VDCKFT T YPSRPDSQV
14 BIEA_HUMAN	Biliverdin reductase A precursor (EC 1.3.1.24) (Biliverdin-IX alpha- reductase).	158 EEE E FGFP A FSGISR
15 CEBA_BOVIN	CCAAT/enhancer binding protein alpha (C/EBP alpha).	27 SSAAFG F PRGAGPSQ
16 CEBA_HUMAN	CCAAT/enhancer binding protein alpha (C/EBP alpha).	27 SSAAFG F PRGAGP A Q
17 CEBE_HUMAN	CCAAT/enhancer binding protein epsilon (C/EBP epsilon).	84 DPRP F AYPPHTFGPD
18	13562153 channel-kinase 1 [Homo sapiens]	1339 QRKE F NPEAGSSSG
19 CLC3_HUMAN	Chloride channel protein 3 (CLC-3).	236 NIFS Y <u>FP</u> KYS T NEA
20 CLC3_HUMAN	Chloride channel protein 3 (CLC-3).	280 EEV S YY F PLKT L WRS
21 CLC6_HUMAN	Chloride channel protein 6 (CLC-6).	223 RKIQEN F YFRSDRD
22 CICL_HUMAN	Chloride channel protein CLC-KB (CLC-K2).	442 ETL S EIF P EGIVAGG
23 CL16_HUMAN	Chloride intracellular channel 6.	638 KYRDFE F PS E MTG I W
24 CETP_HUMAN	Cholesteryl ester transfer protein precursor (Lipid transfer protein 1).	474 LQMDEGF P E H LLVDF
25 CNG4_HUMAN	Cyclic-nucleotide-gated cation channel 4 (CNG channel 4) (CNG-4) (CNG4) (Cyclic nucleotide-gated Cyclic-nucleotide-gated cation channel modulatory subunit).	296 PWK K YQFPQSIDPLT
26 C561_HUMAN	Cytochrome b561 (Cytochrome b-561).	139 GFSF F LF P GASFSLR
27 CX42_HUMAN	Cytochrome c oxidase subunit IV isoform 2, mitochondrial precursor (EC 1.9.3.1) (COX IV-2).	122 WQRVY V FP P KP I LT
28 CP27_HUMAN	Cytochrome P450 27, mitochondrial precursor (EC 1.14.-.-) (Cytochrome P-450C27/25) (Sterol 26-hydroxylase) (Sterol 27-hydroxylase) (Vitamin D(3) 25-hydroxylase) (5-beta-cholestane-3-alpha,7-alpha,12-alpha-triol 27-hydroxylase).	412 EVDGEL F P K N T QVFV F
29	dJ686C3.1.3 (isocitrate dehydrogenase 3 (NAD ⁺) beta (isoform C)) [Homo sapiens]	7 WSSL E PF P VSPSCCF
30 ENP4_HUMAN	Ectonucleoside triphosphate diphosphohydrolase 4 (EC 3.6.1.6) (NTPDase4) (Uridine-diphosphatase) (UDPase) (lysosomal arylarase-like protein of 70 kDa).	500 FHRGFS F PVNYKSLK
31 EAA1_HUMAN	Excitatory amino acid transporter 1 (Sodium-dependent glutamate/aspartate transporter).	77 EVKYF S FP G ELL M RM
32 EAA4_HUMAN	Excitatory amino acid transporter 4 (Sodium-dependent glutamate/aspartate transporter).	85 QIKYF S FP G ELL M RM
33 EAA5_HUMAN	Excitatory amino acid transporter 5 (Retinal glutamate transporter).	46 EISYFQFP G ELL M RM
34 F16P_HUMAN	Fructose-1,6-bisphosphatase (EC 3.1.3.11) (D-fructose-1,6-bisphosphate 1-phosphohydrolase) (FBPase).	258 YGGIFLYP A N O KSPK
35 F16Q_HUMAN	Fructose-1,6-bisphosphatase isozyme 2 (EC 3.1.3.11) (D-fructose-1,6-bisphosphate 1-phosphohydrolase) (FBPase).	259 YGGIFLYP A N O KSPK

36	CST_HUMAN	Galactosylceramide sulfotransferase (EC 2.8.2.11) (GalCer sulfotransferase) (Cerebroside sulfotransferase) (3'- phosphoadenosine-5'-phosphosulfate:GalCer sulfotransferase:galactosylceramide 3'-sulfotransferase).	102 HRLK <u>K</u> AFFP <u>N</u> GRNDFD
37	KLK1_HUMAN	Glandular kallikrein 1 precursor (EC 3.4.21.35) (Tissue kallikrein) (Kidney/pancreas/salivary gland kallikrein).	162 EPEN <u>E</u> SP <u>D</u> DL <u>Q</u> CVD
38	DCE2_HUMAN	Glutamate decarboxylase, 65 kDa isoform (EC 4.1.1.15) (GAD-65) (65 kDa glutamic acid decarboxylase).	131 KVID <u>E</u> HYP <u>N</u> ELL <u>Q</u> EY
39	GSHR_HUMAN	Glutathione reductase, mitochondrial precursor (EC 1.8.1.7) (GR) (GRase).	125 DHADYG <u>G</u> FP <u>S</u> CE <u>G</u> KFN
40	GCSP_HUMAN	Glycine dehydrogenase [decarboxylating], mitochondrial precursor (EC 1.4.4.2) (Glycine decarboxylase) (Glycine cleavage system P- protein).	260 SGVL <u>F</u> QY <u>P</u> D <u>E</u> TKVE
41	GDE_HUMAN	Glycogen debranching enzyme (Glycogen debrancher) [Includes: 4-alpha- glucanotransferase (EC 2.4.1.25) (Oligo-1,4-1,4-glucantransferase); Amylo-alpha-1,6-glucosidase (EC 3.2.1.33) (Amylo-1,6-glucosidase) (Dextrin 6-alpha-D-glucosidase)].	426 VTRYF <u>T</u> FP <u>F</u> EE <u>I</u> D <u>F</u> S
42	KG3A_HUMAN	Glycogen synthase kinase-3 alpha (EC 2.7.1.37) (GSK-3 alpha).	350 NYTE <u>E</u> KFP <u>Q</u> IKAH <u>H</u> PW
43	SYG_HUMAN	Glycyl-tRNA synthetase (EC 6.1.1.14) (Glycine-tRNA ligase) (GlyRS).	601 QRT <u>F</u> SE <u>P</u> AVVAPFK
44	GUAD_HUMAN	Guanine deaminase (EC 3.5.4.3) (Guanase) (Guanine amidase) (Guanine amidohydrolase) (GAH) (p51-nedasrin).	102 WLTKY <u>T</u> FP <u>A</u> EHR <u>F</u> Q <u>N</u>
45	HO1_HUMAN	Heme oxygenase-1 (EC 1.14.99.3) (HO-1).	163 GLAF <u>E</u> TF <u>P</u> N <u>I</u> ASATK
46	HNFB_HUMAN	Hepatocyte nuclear factor 1-beta (HNF-1B) (Variant hepatic nuclear factor 1) (VHNF1).	200 DQLL <u>F</u> LF <u>E</u> F <u>S</u> QQASH
47	HXK4_HUMAN	(Homeoprotein LFB3) (Transcription factor 2) (TCF-2).	146 LGFT <u>F</u> SP <u>V</u> R <u>H</u> EDID
48	HXK3_HUMAN	Hexokinase D (EC 2.7.1.1) (Hexokinase type IV) (HK IV) (HK4) (Glucokinase).	163 LGFS <u>F</u> SP <u>C</u> Q <u>T</u> QLD
49	HXK1_HUMAN	Hexokinase, type I (EC 2.7.1.1) (HK I) (Brain form hexokinase).	598 LGFT <u>F</u> SP <u>C</u> Q <u>T</u> QLD
50	HXK2_HUMAN	Hexokinase, type II (EC 2.7.1.1) (HK II) (Muscle form hexokinase).	598 LGFT <u>F</u> SP <u>C</u> Q <u>N</u> NSLD
51	IKAP_HUMAN	Hexokinase, type II (EC 2.7.1.1) (HK II) (Muscle form hexokinase).	599 FPVR <u>F</u> P <u>P</u> C <u>T</u> Q <u>T</u> ELA
52	IRAP_HUMAN	IkappaB kinase complex-associated protein (IKK complex-associated protein) (p150).	280 QFH <u>S</u> E <u>I</u> P <u>P</u> Y <u>M</u> ANGSL
53	OBRG_HUMAN	Interleukin-1 receptor-associated kinase-2 (EC 2.7.1.-) (IRAK-2).	78 VVS <u>A</u> GF <u>P</u> V <u>I</u> LRVA
54	LIPL_HUMAN	Leptin receptor gene-related protein (OB-R gene related protein) (OB- RGRP).	142 MEEE <u>F</u> NY <u>P</u> LD <u>N</u> V <u>H</u> LL
55	LRP2_HUMAN	Lipoprotein receptor-related protein 2 precursor (Megalin) (Glycoprotein 330) (gp330).	1025 QCG <u>G</u> LF <u>S</u> F <u>P</u> C <u>K</u> NGRCV
56	LOL1_HUMAN	Lysyl oxidase homolog 1 precursor (EC 1.4.3.-) (Lysyl oxidase-like protein 1) (LOL).	173 YPQQ <u>F</u> P <u>P</u> Y <u>Q</u> AP <u>F</u> V <u>S</u> Q
57	MDHC_HUMAN	Malate dehydrogenase, cytoplasmic (EC 1.1.1.37).	281 DDL <u>Y</u> SP <u>V</u> V <u>I</u> KNKT
58	MUTA_HUMAN	Methylmalonyl-CoA mutase, mitochondrial precursor (EC 5.4.99.2) (MCM).	227 VRNT <u>Y</u> IF <u>P</u> PE <u>P</u> SMKI
59	RT29_HUMAN	Mitochondrial 28S ribosomal protein S29 (S29mt) (MRP-S29) (Death- associated protein 3) (DAP-3).	113 KNT <u>S</u> FAY <u>P</u> A <u>I</u> R <u>Y</u> LL
60	14141575	(Ionizing radiation resistance conferring protein).	2 HGQT <u>E</u> TF <u>P</u> D <u>L</u> PE <u>K</u> D
61	MOT1_HUMAN	Monocarboxylate transporter 1 (MCT 1).	30 IGF <u>S</u> Y <u>A</u> FP <u>K</u> S <u>T</u> V <u>F</u> F
62	MOT2_HUMAN	Monocarboxylate transporter 2 (MCT 2).	29 TGF <u>A</u> Y <u>G</u> FP <u>K</u> A <u>V</u> S <u>V</u> F
63	MOT3_HUMAN	Monocarboxylate transporter 3 (MCT 3).	32 TGF <u>S</u> Y <u>A</u> FP <u>K</u> A <u>V</u> S <u>V</u> F
64	MOT4_HUMAN	Monocarboxylate transporter 4 (MCT 4) (MCT 3).	

65	MYHD_HUMAN	Myosin heavy chain, skeletal muscle, extraocular (MyHC-eo). N-acetylglucosaminyl-phosphatidylinositol biosynthetic protein (GlcNAc-PI synthase protein)	306	STNP <u>FDFPFVSSQEV</u>
66	PIGA_HUMAN	(Phosphatidylinositol-glycan biosynthesis, class A protein) (PIG-A). NADH-ubiquinone oxidoreductase B18 subunit (EC 1.6.5.3) (EC 1.6.99.3) (Complex I-B18) (CI-B18)	37	MVSD <u>FFYPNMGGVES</u>
67	NB8M_HUMAN	(Cell adhesion protein SQM1).	24	FPP <u>DYGFPERKEREM</u>
68	N12M_HUMAN	NADH-ubiquinone oxidoreductase B22 subunit (EC 1.6.5.3) (EC 1.6.99.3) (Complex I-B22) (CI-B22).	75	HPQ <u>QYIFPDSPGGTS</u>
69	MAOX_HUMAN	NADP-dependent malic enzyme (EC 1.1.1.40) (NADP-ME) (Malic enzyme 1).	452	GNNS <u>SYVFPGVALGVV</u>
70	NPH4_HUMAN	Nephrocystin 4 (Nephroretinin).	669	TFQ <u>QFYRFPPATPTRL</u>
71	NCO2_HUMAN	Nuclear receptor coactivator 2 (NCoA-2) (Transcriptional intermediary factor 2).	1295	NAQ <u>QFPEPPNYGISQ</u>
72	NCR1_HUMAN	Nuclear receptor co-repressor 1 (N-CoR1) (N-CoR). Nuclear receptor co-repressor 2 (N-CoR2) (Silencing mediator of retinoic acid and thyroid hormone receptor) (SMRT) (SMRTE) (Thyroid-, retinoic-acid-receptor-associated co-repressor) (T3 receptor-associating factor) (TRAC) (CTG repeat protein 26).	23	HSV <u>QYQTEPNTRHQQE</u>
73	NCR2_HUMAN	Nuclear receptor ROR-gamma (Nuclear receptor RZR-gamma).	2160	PAP <u>LYSFPPGASC</u> CPVL
74	RORG_HUMAN	Oligosaccharyl transferase STT3 subunit homolog (B5) (Integral membrane protein 1) (TMC).	540	PPSP <u>SFFPMNPGGWS</u>
75	STT3_HUMAN	Pantothenate kinase 4 (EC 2.7.1.33) (Pantothenic acid kinase 4) (hPanK4).	365	QLLV <u>FMFPVGLYYCF</u>
76	PNK4_HUMAN	Peroxisome proliferator activated receptor alpha (PPAR-alpha).	513	CLNE <u>FNFPDPYSKVK</u>
77	PPAR_HUMAN	Peroxisome proliferator activated receptor delta (PPAR-delta) (PPAR-beta) (Nuclear hormone receptor 1) (NUC1).	417	PDD <u>IFLPKLLQKMA</u>
78	PPAS_MOUSE	Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit, delta isoform (EC 2.7.1.153) (PI3-kinase p110 subunit delta) (PtdIns- 3-kinase p110) (PI3K) (p110delta).	389	PDS <u>SQLFPKLLQKMA</u>
79	P11D_HUMAN	Phosphatidylinositol-glycan biosynthesis, class O protein (PIG-O).	581	ELL <u>DEFSPFDCHVGSF</u>
80	PIGO_HUMAN	Phosphodiesterase A' subunit [Homo sapiens]	190	FSK <u>AFFFPSSFNVRDL</u>
81	940231		366	ADEY <u>FTFPKGFPVDET</u>
82	KPRA_HUMAN	Phosphoribosyl pyrophosphate synthetase-associated protein 1 (PRPP synthetase-associated protein 1) (39 kDa phosphoribosylpyrophosphate synthetase synthetase-associated protein) (PAP39).	140	IQGF <u>FSFPVDNLRAS</u>
83	P1T1_HUMAN	Pituitary-specific positive transcription factor 1 (Pit-1) (Growth hormone factor 1) (GHF-1).	75	TPCL <u>YKFPDHTLSHG</u>
84	PKHD_HUMAN	Polycystic kidney and hepatic disease 1 precursor (Fibrocystin) (Polyductin) (Trigmin).	3321	DKN <u>KFYFPSSLQPRKD</u>
85	PKHD_HUMAN	Polycystic kidney and hepatic disease 1 precursor (Fibrocystin) (Polyductin) (Trigmin).	2512	NVA <u>FFPFPHAAILED</u>
86	PKHD_HUMAN	Polycystic kidney and hepatic disease 1 precursor (Fibrocystin) (Polyductin) (Trigmin).	2370	RYGL <u>FVYPKFQPPWD</u>
87	P1L1_HUMAN	Polycystic kidney disease 1-like 1 protein (Polycystin 1L1).	2687	PGLL <u>HFPRRSQKDC</u>
88	PKDR_HUMAN	Polycystic kidney disease and receptor for egg jelly related protein precursor (PKD and REJ homolog).	1880	GYAL <u>YFFPEQQRFNS</u>
89	PKD1_HUMAN	Polycystin 1 precursor (Autosomal dominant polycystic kidney disease protein 1).	3060	SHVR <u>FVFPPEPTADVN</u>
90	PAGT_HUMAN	Polypeptide N-acetylgalactosaminyltransferase (EC 2.4.1.41) (Protein- UDP acetylgalactosaminyltransferase) (UDP-GalNAc:polypeptide, N- acetylgalactosaminyltransferase) (GalNAc-T1).	348	KATPY <u>TFFPGGTGQII</u>
91	DP1_HUMAN	Polyposis locus protein 1 (TB2 protein).	56	NLIG <u>FGYPAYISKA</u>

92	CIK1_HUMAN	Potassium voltage-gated channel subfamily A member 1 (Potassium channel Kv1.1) (HUK1) (HK2).	153	VWLLFEYPESSGGPAR
93	CIK5_HUMAN	Potassium voltage-gated channel subfamily A member 5 (Potassium channel Kv1.5) (HK2) (HPCN1).	236	VWLIFEYPESSGSAR
94	KCH7_HUMAN	Potassium voltage-gated channel subfamily H member 7 (Ether-a-go-go related gene potassium channel 3) (HERG-3) (Ether-a-go-go related protein 3) (Eag related protein 3).	156	KFFGFKEFPGLRVLTY
95	S24C_HUMAN	Protein transport protein Sec24C (SEC24-related protein C).	942	ETNVFFYPRLLPLTK
96	60078266	rab escort protein-2 [Mus musculus]	370	GNTPFIFFPLYGHGEI
		Renal sodium-dependent phosphate transport protein 2 (Sodium/phosphate cotransporter 2) (Renal Na(+) -dependent (Na(+))/F _i cotransporter 2) (Renal sodium-phosphate transport protein 2) (Renal Na(+) -dependent phosphate cotransporter 2).	43	GTSAYAEPSSLGPVAL
		Retinoic acid receptor alpha (RAR-alpha).	22	PPYAFFFPMLGGLS
		Retinoic acid receptor gamma-1 (RAR-gamma-1).	23	AGFPFAFPGALRGSP
		Retinoic acid receptor gamma-1 (RAR-gamma-1).	370	PSQPYMFPRMLMKIT
		Retinoic acid induced protein 1 [Homo sapiens]	1830	PISLFSFPPPLQQF
		101 120537933 retinoid-acid binding Ig-like lectin 5 precursor (Siglec-5) (Obesity- binding protein 2) (OB binding protein-2) (OB-BP2) (CD33 antigen-like 2) (CD170 antigen).	39	VPCSFESYPWRSWYSS
		Sialic acid binding glycine transporter 1 (GlyT-1) (GlyT-1).	120	GGGAFMFPYFIMLIF
		Sodium- and chloride-dependent glycine transporter 1 (GlyT-1) (GlyT-1).	279	IGPLFFFPLLYMIFQ
		Sodium/bile acid cotransporter (Na(+))/bile acid cotransporter (Na(+))/taurocholate transport protein).	835	PRSS2AEPPLSLAKAG
		(Sodium/taurocholate cotransporting polypeptide).	525	EYGSYRFPWAAELLG
		Sodium/hydrogen exchanger 5 (Na(+)/H(+) exchanger 5) (NHE-5).		
		Sodium-dependent proline transporter.		
		Solute carrier family 21 member 11 (Sodium-independent organic anion transporter D) (Organic anion transporting polypeptide D) (OATP-D) (Organic anion transporter polypeptide related protein 3) (OATP-RP3) (PGE1 transporter).	279	SLLMFGFPQSLPPHS
		Synaptotagmin X (SytX).	296	FDELFQFPVWYDQLS
		Synaptotagmin XI (SytXI).	163	FSVVDYNFPKKALWVT
		Thyroid hormone receptor-associated protein complex 240 kDa component (Trap240) (Activator-recruited cofactor 250 kDa component) (ARC250).	703	GDEEEFLFPDKKDRQN
		Thyroid hormone receptor-associated protein complex 240 kDa component (Trap240) (Activator-recruited cofactor 250 kDa component) (ARC250).	614	AWKYYKFPKKKDVEF
		Thyroid receptor interacting protein 12 (TRIP12).	1536	KTCPFFFDPDTRQML
		Thyroid receptor interacting protein 12 (TRIP12).	570	YTVRFTFPDPPLSP
		110 T240_HUMAN	767	GFGSFRFPSSGNQGGA
		TNalpha-inducible ATP-binding protein [Homo sapiens]	498	FTMDFCFFPSPLQAF
		111 T240_HUMAN	1518	YILDEQYPPFSAVQAF
		Transitional endoplasmic reticulum ATPase (TER ATPase) (15S Mg(2+)- ATPase p97 subunit)		
		(Valosin containing protein) (VCP) [Contains: Valosin].		
		Tubby related protein 2 (Tubby-like protein 2).		
		Tubby superfamily protein.		
		Tumor necrosis factor receptor superfamily member 11B precursor (Osteoprotegerin) (Osteoclastogenesis inhibitory factor).	351	HSKTYHFPKTVTQSL
		114 TERA_HUMAN		
		115 TUL2_HUMAN		
		116 TUSP_HUMAN		
		117 T11B_HUMAN		

118	T13X_HUMAN	Tumor necrosis factor receptor superfamily member 13B (Transmembrane activator and CAML interactor).
119	JAK2_HUMAN	Tyrosine-protein kinase JAK2 (EC 2.7.1.112) (Janus kinase 2) (JAK-2).
120	UDBF_HUMAN	UDP-glucuronosyltransferase 2B15 precursor, microsomal (EC 2.4.1.17) (UDPGT) (UDPGT-3) (HLUG4).
121	V1BR_HUMAN	Vasopressin V1b receptor (V1bR) (AVPR V1b) (Vasopressin V3 receptor) (AVPR V3) (Antidiuretic hormone receptor 1b).
122	16904210	very large G protein-coupled receptor 1 [Mus musculus]
123	WRN_HUMAN	Werner syndrome helicase.
124	WFS1_HUMAN	Wolframin.
125	ZN44_HUMAN	Zinc finger protein 44 (Zinc finger protein K0X7) (Gonadotropin inducible transcription repressor-2) (GIOT-2).

TABLE 6: NEUROPATHIES

Accession Code	Target Description	Amino Acid	Target Sequence
1 5H1B_HUMAN	5-hydroxytryptamine 1B receptor (5-HT-1B) (Serotonin receptor) (5-HT-1D-beta) (Serotonin 1D beta receptor) (S12).	213	TVGAFYFPTLLIAL
2 5H2C_HUMAN	5-hydroxytryptamine 2C receptor (5-HT-2C) (Serotonin receptor) (5HT-1C).	42	DGGGRFKFPDPGVQNWP
3 5HT3_HUMAN	5-hydroxytryptamine 3 receptor (5-HT-3) (Serotonin-gated ion channel receptor) (5-HT3R).	158	SLDIYNEFPFDVQNCNS
4 AUT2_HUMAN	Autism susceptibility gene 2 protein.	1057	GEREFYPYSFHWDPI
5 BIR1_HUMAN	Baculoviral IAP repeat-containing protein 1 (Neuronal apoptosis inhibitory protein).	649	CAHWFQYPPFDPSFDD
6 BRA1_HUMAN	Brain link protein-1 precursor.	318	GVRSEGFPRPQQAAY
7 BAI1_HUMAN	Brain-specific angiogenesis inhibitor 1 precursor.	341	ELQQEGEPAPQTGDP
8 CBL2_HUMAN	Cdk5 and abl enzyme substrate 2 (Interactor with cdk3 2) (Ik3-2).	241	SYAKFLYPTNALVTH
9 DYHB_HUMAN	Ciliary dynein heavy chain 1 (Axonemal beta dynein heavy chain 1).	2649	TVFAFNFPSSLDALNT
10 FMO5_HUMAN	Dimethylamine monoxygenase [N-oxide forming] 5 (EC 1.14.13.8) (Hepatic flavin-containing monooxygenase 5) (FMO 5) (Dimethylamine oxidase 5).	329	CGYSFDEFPLEDSVK
11 DIS1_HUMAN	Disrupted in schizophrenia 1 protein.	61	VGTLFRFPGGVSSEE
12 DOPO_MOUSE	Dopamine beta-monoxygenase precursor (EC 1.14.17.1) (Dopamine beta-hydroxylase) (DBH).	309	GAKAFYYPKAEAGVPF
13 DSCA_HUMAN	Down syndrome cell adhesion molecule precursor (CHD2).	80	TLQIIFPPPPSSFSFSTL
14 DSCA_HUMAN	Down syndrome cell adhesion molecule precursor (CHD2).	598	FIQPFEEFPRFSIGQR
15 DSR3_HUMAN	Down syndrome critical region protein 3 (Down syndrome critical region protein A).	89	TEIPFEFPLHLKGNK
16 DYSF_HUMAN	Dysferlin (Dystrophy associated fer-1-like protein) (Fer-1 like protein 1).	1867	FNWWRFIFPPFDYLPAE
17 EPA7_HUMAN	Ephrin type-A receptor 7 precursor (EC 2.7.1.112) (Tyrosine-protein kinase receptor EHK-3) (Eph homology kinase-3) (Receptor protein-tyrosine kinase HEK11).	596	LYFFHFKFPGTKTYID
18 ERC6_HUMAN	Excision repair protein ERCC-6 (Cockayne syndrome protein CSB).	687	SLFDEIIPGKLGTLP

19	EAA3_HUMAN	Excitatory amino acid transporter 3 (Sodium-dependent glutamate/aspartate transporter) (Excitatory amino-acid carrier 1) (Neuronal and epithelial glutamate transporter).	48	EKFYFAFPGEIILMRM
20	FXGA_HUMAN	Forkhead box protein G1A (Forkhead-related protein FKH2) (Transcription factor BF-2) (Brain factor 2) (BF2) (HFK2).	391	GQTSYFFPHPHPSM
21	FMR2_HUMAN	Fragile X mental retardation 2 protein (Protein FMR-2) (FMR2P) (Ox19 protein) (Fragile X E mental retardation syndrome protein).	639	STDEF T WP K PNITSS
22	FMR2_HUMAN	Fragile X mental retardation 2 protein (Protein FMR-2) (FMR2P) (Ox19 protein) (Fragile X E mental retardation syndrome protein).	193	LEDFFVYPAEQPQIG
23	FCMD_HUMAN	Fukutin precursor (Fukuyama-type congenital muscular dystrophy protein).	388	KKF KY LF P KFT L CWT
24	GAE_HUMAN	Gamma-aminobutyric-acid receptor epsilon subunit precursor (GABA(A) receptor).	212	SFSSFSY P ENEMIYK
25	GAAT_HUMAN	Gamma-aminobutyric-acid receptor theta subunit precursor (GABA(A) receptor).	609	KWSREL F PLA G LFN
26	SGCG_HUMAN	Gamma-sarcoglycan (Gamma-SG) (35 kDa dystrophin-associated glycoprotein) (35DAG).	81	GESEELF P LYAKEIH
27	KG3A_HUMAN	Glucose synthase kinase-3 alpha (EC 2.7.1.37) (GSK-3 alpha).	350	NYTEEFKEPQIAHPW
28		Kainate receptor subunit KA2a [Homo sapiens]	945	DASSFFFPISSCPP
29	OPRK_HUMAN	Kappa-type opioid receptor (KOR-1).	340	CFRDFCFPLKMRMER
30		Kinesin-related protein HASH [Mus musculus]	46	NTRD M FP G P N QMSG
31	MGR1_HUMAN	Metabotropic glutamate receptor 1 precursor (mGluR1).	3	GLL L FFFPAIFLEVS
32		Myoxygenase X [Homo sapiens]	9	RYPD E SFPYFPQDYF
33	MY15_HUMAN	Myosin XV (Unconventional myosin-15).	751	RGAAG G FP G ASP R S
34	MTR2_HUMAN	Myotubularin-related protein 2.	178	NLMKYA F FP V NNLPL
35		Myotonic dystrophy kinase-related Cdc42-binding kinase [Rattus norvegicus]	297	HKERFQ F PTQ V TDVS
36	NCA2_HUMAN	Neurogenin 1 (Neurogenic differentiation factor 3) (NeuroD3) (Neurogenic basic-helix-loop-helix antigen).	425	TCEVFAYPSATISW F
37	NTC3_HUMAN	Neurogenin 1 (Neurogenic basic-helix-loop-helix antigen).	1618	ERLD F P Y PLR D VRGE
38	NGN1_HUMAN	Neurotensin receptor type 1 (NT-R-1) (High-affinity levocabastine- insensitive neurotensin receptor (NTRH)).	213	GDPVSE S PLPKD L L
39	NTR1_HUMAN	Neutral amino acid transporter B(0) (ATB(0)) (Sodium-dependent neutral amino acid transporter type 2) (RD114/simian type D retrovirus receptor) (Baboon M7 virus receptor).	241	TMSE I FP M V V ISVL
40	AAAT_HUMAN	Olfactory receptor 10A3 (HTPCR X 12).	85	RLSAF V FPGE L LLRL
41	OAA3_HUMAN	Olfactory receptor 10A5 (HP3) (Olfactory receptor-like protein JCG6).	160	TTW V ESFP F CGGPNEI
42	OAA5_HUMAN	Olfactory receptor 51B2 (HOR5'beta3).	161	TTW L FSFP F CGTNK V
43	OXB2_HUMAN	Olfactory receptor 51B4 (HOR5'beta1).	275	SYIYFLFPPLMN N PVI
44	OXB4_HUMAN	Olfactory receptor 5111 (HOR5'beta11).	155	SYVHFLFP P FW K R L P
45	OXI1_HUMAN	Olfactory receptor 6B1 (Olfactory receptor 7-3) (OR7-3).	203	ALV I FLFP L FT V LS
46	O6B1_HUMAN	Pannexin 1.	187	SESHFKY P IVEQY L K
47	PAN1_HUMAN	Reelin precursor (EC 3.4.21.-).	1894	LMDEFYFPQ T TLIF
48	RELN_HUMAN	Reelin precursor (EC 3.4.21.-).	1967	EDNWFFY P GGNIGLY
49	RELN_HUMAN	Sacsin.	1944	NCTM F REPLRNAEMA
50	SACS_HUMAN			

51	SAD1_HUMAN	SAM domain and HD domain-containing protein 1 (Dendritic cell-derived IFNG-induced protein (DCIP) (Monocyte protein 5) (MOP-5)).
52	KPT3_HUMAN	Serine/threonine protein kinase PCTAIRE-3 (EC 2.7.1.-).
53	SAMP_HUMAN	Serum amyloid P-component precursor (SAP) (9.5S alpha-1-glycoprotein). SH3 and multiple ankyrin repeat domains protein 2 (Shank2) (Proline-rich synapse associated protein 1) (ProSAP1) (Cortactin-binding protein 1) (CortBP1) (GKAP/SAPAP interacting protein (SPANK-3)).
54	SHK2_RAT	similar to dj309K20.4 (KIAA0765, putative brain nucleolar targeted protein (HRHFB2091, RNA similar to dj309K20 recognition motif (RNP, RRM or RBD domain) containing protein)) (Mus musculus)
55		20073220 Similar to Per1 interacting protein [Mus musculus]
56		20071167 Similar to Per1 interacting protein [Mus musculus]
57	S6A1_HUMAN	Sodium- and chloride-dependent GABA transporter 1.
58	SX14_HUMAN	Transcription factor SOX-14.
59	ROR1_HUMAN	Tyrosine-protein kinase transmembrane receptor ROR1 precursor (EC 2.7.1.112) (Neurotrophic tyrosine kinase, receptor-related 1).
60	ROR1_HUMAN	Tyrosine-protein kinase transmembrane receptor ROR1 precursor (EC 2.7.1.112) (Neurotrophic tyrosine kinase, receptor-related 1).
61	ROR2_HUMAN	Tyrosine-protein kinase transmembrane receptor ROR2 precursor (EC 2.7.1.112) (Neurotrophic tyrosine kinase, receptor-related 2).
62	WS14_HUMAN	Williams-Beuren syndrome chromosome region 14 protein (WS basic-helix-loop-helix leucine zipper protein) (WS-bHLH) (Mlx interactor).
63	WFS1_HUMAN	Wolframin.

TABLE 7: MISCELLANEOUS DEF DOMAIN-CONTAINING PROTEINS

Accession Code	Target Description	Amino Acid	Target Sequence
1	15425674 Per1 interacting protein of the suprachiasmatic nucleus [Rattus norvegicus]	1023	SLNPERREPKEAASLF
2	PER1_HUMAN	922	VLPNYLFPTPSSPYY
3	PER2_HUMAN	907	MLPSYSFSPSGTPNLP
4	PER3_HUMAN	843	PYPAFPPFYLDTFMT
5	AK11_HUMAN	661	EVCAQFSYYPQTFASQ
6	AKA3_HUMAN	490	SDISEEYEPEDIGNLS

Claims

1. A method for identifying therapeutic compounds, said method comprising:
 - (i) providing test cells, wherein said test cells express a target protein containing a DEF domain and a MAP kinase,
 - (ii) culturing said test cells in the presence of a growth factor, cytokine, tumor promoter, or oncogene,
 - (iii) contacting said cells with a candidate compound, and
 - (iv) assessing the binding of said MAP kinase to said target protein, wherein a candidate compound that inhibits said binding is a therapeutic compound.
2. A method for identifying a therapeutic compound, said method comprising:
 - (i) providing a target protein comprising a DEF domain, a MAP kinase, and a candidate compound,
 - (ii) mixing said target protein, said MAP kinase, and said candidate compound,
 - (iii) measuring the binding of said MAP kinase to said target protein, and
 - (iv) identifying said candidate compound as a therapeutic compound, wherein said candidate compound inhibits said binding, compared to the binding of said MAP kinase to said target protein in the absence of said candidate compound.
3. The method of claim 1 or 2, wherein said DEF domain comprises the amino acid sequence F/Y-X-F/Y-X (SEQ ID NO: 28).
4. The method of claim 3, wherein said DEF domain comprises the amino acid sequence F/Y-X-F/Y-P (SEQ ID NO: 29).
5. The method of claim 4, wherein said DEF domain comprises the amino acid sequence F-X-F-P (SEQ ID NO: 1).

6. The method of claim 1, wherein said test cells are selected from the group consisting of fibroblasts, a primary cell line, an immortalized cell line, and a tumor-derived cell line.

7. The method of claim 1, wherein said test cells are human.

8. The method of claim 1, wherein said growth factor, cytokine, tumor promoter, or oncogene is selected from the group consisting of epidermal growth factor (EGF), transforming growth factor α , heparin-binding-like EGF, heregulin, amphiregulin, epiregulin, cripto, PDGF-AA, PDGF-BB or PDGF-CC, insulin, insulin-like growth factors, fibroblast growth factors, colony stimulating factor, heaptocyte growth factor, a chemokine, an interleukin, lysophosphatidic acid, a phorbol ester, okadaic acid, microcystin, vanadate, hydrogen peroxide, calyculin A, Erb2/neu, sis, kit, Ras, Raf, PI3-kinase, and PTEN.

9. The method of claim 1 or 2, wherein said MAP kinase is extracellular signal-regulated kinase 1/2 (ERK1/2).

10. The method of claim 1 or 2, wherein said target protein is a Fos, Myc, or Jun family protein.

11. The method of claim 10, wherein said target protein is c-Fos.

12. The method of claim 1 or 2, wherein said therapeutic is useful for the treatment of cancer and said target protein comprises the sequence of a protein identified in Tables 1 or 2.

13. The method of claim 1 or 2, wherein said therapeutic is useful for the

treatment of a cardiovascular disorder and said target protein comprises the sequence of a protein identified in Table 3.

14. The method of claim 1 or 4, wherein said therapeutic is useful for the treatment of an inflammatory disorder, and said target protein comprises the sequence of a protein identified in Table 4.

15. The method of claim 1 or 2, wherein said therapeutic is useful for the treatment of a metabolic disorder, and said target protein comprises the sequence of a protein identified in Table 5.

16. The method of claim 1 or 2, wherein said therapeutic is useful for the treatment of a neuropathy or a behavioral disorder, and said target protein comprises the sequence of a protein identified in Table 6.

17. The method of claim 1 or 2, wherein said therapeutic is useful for the treatment of a sleep disorder, and said target protein comprises the sequence of a protein identified in Table 7.

18. The method of claim 1, wherein said target protein is c-Fos and step (vi) comprises assessing the phosphorylation of T325 or T331.

19. The method of claim 18, wherein said step (vi) comprises an antibody that specifically binds to phospho-T-325 c-Fos.

20. The method of claim 2, wherein said target protein further comprises a fluorescent moiety.

21. The method of claim 20, wherein said fluorescent moiety is fluorescein.
22. A method for treating a cancer in a mammal, said method comprising administering a therapeutically effective amount of a compound that inhibits the binding of a MAP kinase to the DEF domain of a target protein.
23. The method of claim 22, wherein said MAP kinase is ERK1/2.
24. The method of claim 22, wherein said target protein is an immediate early gene.
25. The method of claim 22, wherein said target protein is selected from the group consisting of c-Fos, Fra-1, Fra-2, c-Myc, N-Myc, JunD, JunB, and c-Jun.
26. The method of claim 25, wherein said target protein is c-Fos.
27. The method of claim 22, wherein said target protein is a protein identified in Tables 1 or 2.
28. The method of claim 22, wherein said cancer is selected from the group consisting of leukemia, Hodgkin's disease lymphoma, non-Hodgkin's disease lymphoma, fibrosarcoma, liposarcoma, osteogenic sarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, Wilm's tumor, cervical cancer, uterine cancer, testicular cancer, small cell lung carcinoma, non-small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, schwannoma, meningioma, melanoma, neuroblastoma, and retinoblastoma.

29. The method of claim 22, wherein said DEF domain comprises the amino acid sequence F/Y-X-F/Y-X (SEQ ID NO: 28).
30. The method of claim 22, wherein said DEF domain comprises the amino acid sequence F/Y-X-F/Y-P (SEQ ID NO: 29).
31. The method of claim 22, wherein said DEF domain comprises the amino acid sequence F-X-F-P (SEQ ID NO: 1).
32. A method for treating cardiovascular disease in a mammal, said method comprising administering a therapeutically effective amount of a compound that inhibits the binding of a MAP kinase to the DEF domain of a target protein.
33. The method of claim 32, wherein said MAP kinase is ERK1/2.
34. The method of claim 32, wherein said target protein is a protein identified in Table 3.
35. The method of claim 32, wherein said cardiovascular disease is selected from the group consisting of ischemic heart disease, ventricular heart failure, cardiac hypertrophy, hypertension, and atherosclerosis.
36. The method of claim 32, wherein said DEF domain comprises the amino acid sequence F/Y-X-F/Y-X (SEQ ID NO: 28).
37. The method of claim 32, wherein said DEF domain comprises the amino acid sequence F/Y-X-F/Y-P (SEQ ID NO: 29).

38. The method of claim 32, wherein said DEF domain comprises the amino acid sequence F-X-F-P (SEQ ID NO: 1).

39. A method for treating an inflammatory disorder in a mammal, said method comprising administering a therapeutically effective amount of a compound that inhibits the binding of a MAP kinase to the DEF domain of a target protein.

40. The method of claim 39, wherein said MAP kinase is ERK1/2.

41. The method of claim 39, wherein said target protein is a protein identified in Table 4.

42. The method of claim 39, wherein said inflammatory disorder is selected from the group consisting of anaphylaxis, septic shock, allergic rhinitis, asthma, atopic dermatitis, and food allergies. Examples of autoimmune disorders include, but are not limited to, type 1 insulin-dependent diabetes mellitus, inflammatory bowel disease, Crohn's disease, ulcerative colitis, dermatitis, meningitis, thrombotic thrombocytopenic purpura, Sjögren's syndrome, encephalitis, uveitis, leukocyte adhesion deficiency, rheumatoid and other forms of immune arthritis, rheumatic fever, Reiter's syndrome, psoriatic arthritis, progressive systemic sclerosis, primary biliary cirrhosis, pemphigus, pemphigoid, necrotizing vasculitis, myasthenia gravis, multiple sclerosis, lupus erythematosus, polymyositis, sarcoidosis, granulomatosis, vasculitis, pernicious anemia, CNS inflammatory disorder, antigen-antibody complex mediated diseases, autoimmune hemolytic anemia, Hashimoto's thyroiditis, Graves disease, habitual spontaneous abortions, Reynard's syndrome, glomerulonephritis, dermatomyositis, chronic active hepatitis, celiac disease, autoimmune complications of AIDS, atrophic gastritis, ankylosing spondylitis and Addison's disease.

43. The method of claim 39, wherein said DEF domain comprises the amino acid sequence F/Y-X-F/Y-X (SEQ ID NO: 28).

44. The method of claim 39, wherein said DEF domain comprises the amino acid sequence F/Y-X-F/Y-P (SEQ ID NO: 29).

45. The method of claim 39, wherein said DEF domain comprises the amino acid sequence F-X-F-P (SEQ ID NO: 1).

46. A method for treating a metabolic disorder in a mammal, said method comprising administering a therapeutically effective amount of a compound that inhibits the binding of a MAP kinase to the DEF domain of a target protein.

47. The method of claim 46, wherein said MAP kinase is ERK1/2.

48. The method of claim 46, wherein said target protein is a protein identified in Table 5.

49. The method of claim 46, wherein said metabolic disorder is selected from the group consisting of diabetes, obesity, jaundice, polycystic kidney and hepatic disease, pancreatitis, Graves' disease, and Werner's syndrome.

50. The method of claim 46, wherein said DEF domain comprises the amino acid sequence F/Y-X-F/Y-X (SEQ ID NO: 28).

51. The method of claim 46, wherein said DEF domain comprises the amino acid sequence F/Y-X-F/Y-P (SEQ ID NO: 29).

52. The method of claim 46, wherein said DEF domain comprises the amino acid sequence F-X-F-P (SEQ ID NO: 1).

53. A method for treating a neuropathy or behavioral disorder in a mammal, said method comprising administering a therapeutically effective amount of a compound that inhibits the binding of a MAP kinase to the DEF domain of a target protein.

54. The method of claim 53, wherein said MAP kinase is ERK1/2.

55. The method of claim 53, wherein said target protein is a protein identified in Table 6.

56. The method of claim 53, wherein said neuropathy or behavioral disorder is selected from the group consisting of diabetic neuropathy, muscular dystrophy, Williams Beuren's Syndrome, psychosis, schizophrenia, autism, Down's Syndrome, Parkinson's Disease, Alzheimer's Disease, epilepsy, Cockayne syndrome, bipolar disorders, depression, and opiate addiction.

57. The method of claim 53, wherein said DEF domain comprises the amino acid sequence F/Y-X-F/Y-X (SEQ ID NO: 28).

58. The method of claim 53, wherein said DEF domain comprises the amino acid sequence F/Y-X-F/Y-P (SEQ ID NO: 29).

59. The method of claim 53, wherein said DEF domain comprises the amino acid sequence F-X-F-P (SEQ ID NO: 1).

60. A method for treating a sleep disorder in a mammal, said method comprising administering a therapeutically effective amount of a compound that inhibits the binding of a MAP kinase to the DEF domain of a target protein.

61. The method of claim 60, wherein said MAP kinase is ERK1/2.

62. The method of claim 60, wherein said target protein is a protein identified in Table 7.

63. The method of claim 60, wherein said sleep disorder is selected from the group consisting of advanced sleep phase disorder, delayed sleep phase disorder, insomnia, and narcolepsy.

64. The method of claim 60, wherein said DEF domain comprises the amino acid sequence F/Y-X-F/Y-X (SEQ ID NO: 28).

65. The method of claim 60, wherein said DEF domain comprises the amino acid sequence F/Y-X-F/Y-P (SEQ ID NO: 29).

66. The method of claim 60, wherein said DEF domain comprises the amino acid sequence F-X-F-P (SEQ ID NO: 1).

67. An antibody that specifically binds to phospho-T-325 c-Fos.

68. The antibody of claim 67, wherein said antibody is polyclonal.

69. The antibody of claim 67, wherein said antibody is monoclonal.

70. A pharmaceutical formulation comprising a therapeutic compound identified by the method of claim 1 or 2 and a pharmaceutically acceptable carrier.

INHIBITORS OF THE MAP KINASE PATHWAY

Abstract of the Disclosure

MAP kinases (e.g., ERK1/2) phosphorylate a variety of target proteins including, for example, several immediate early gene products (e.g., Fos, Myc, and Jun family proteins). Certain phosphorylation reactions require binding of the MAP kinase to the DEF domain of the target protein. Inhibitors that block this interaction may be useful therapeutics for human disease, including as antineoplastic agents. Also disclosed are screening assays useful for identifying compounds that inhibit the MAP kinase-DEF domain interaction.

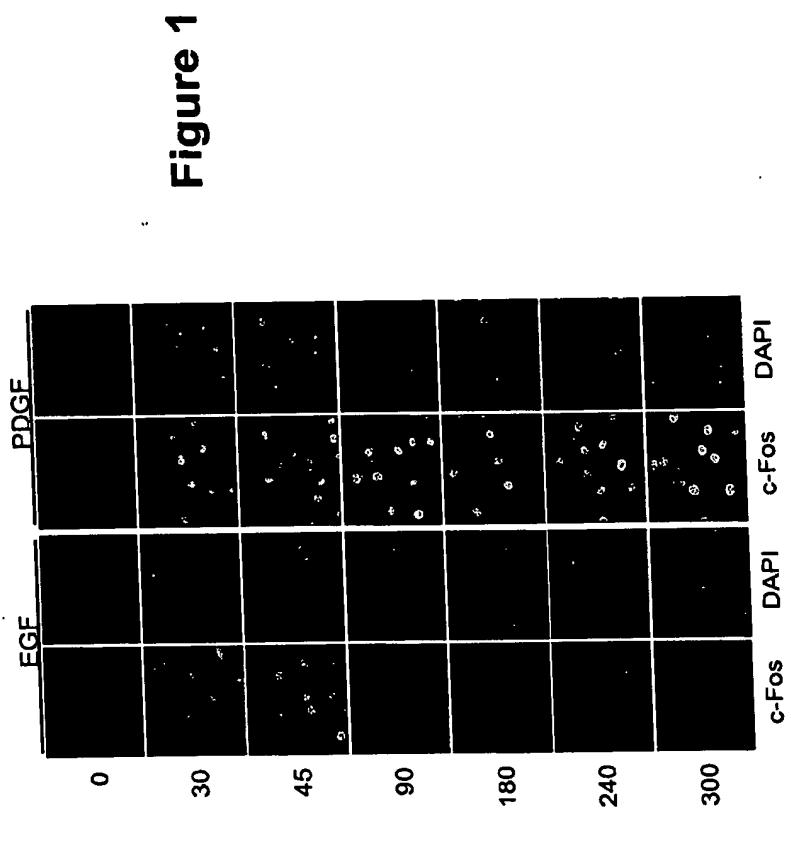
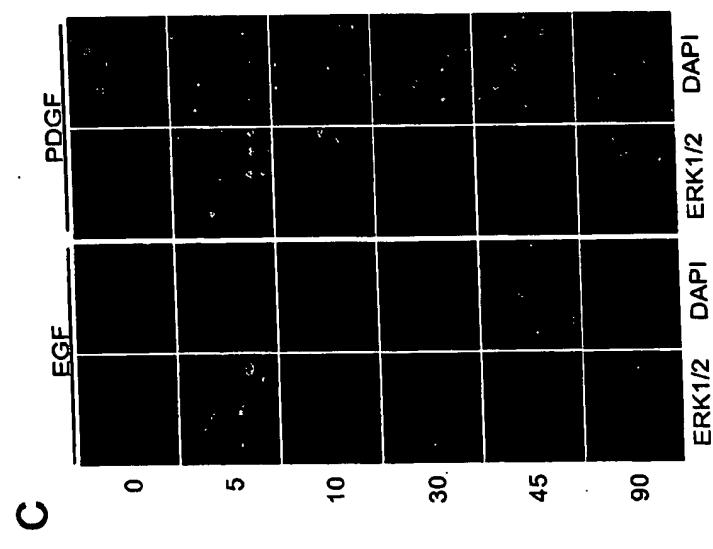
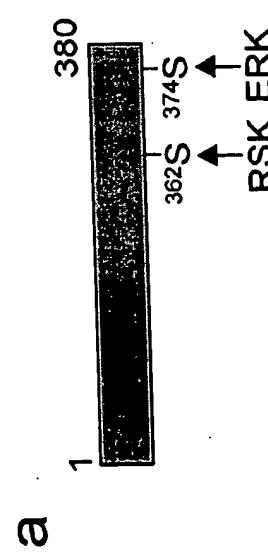
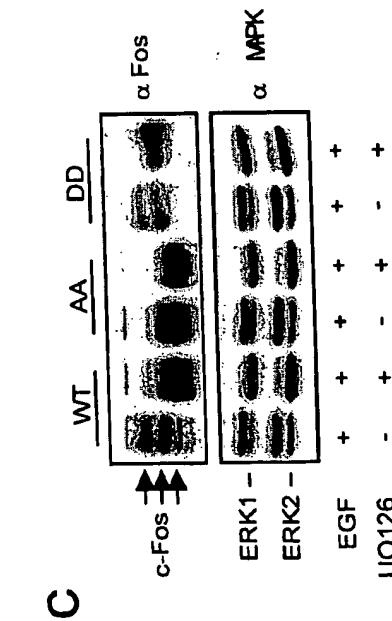
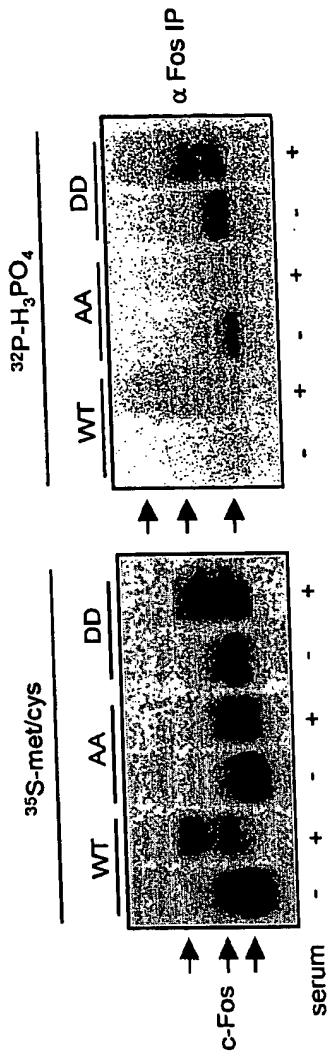


Figure 1



b



d

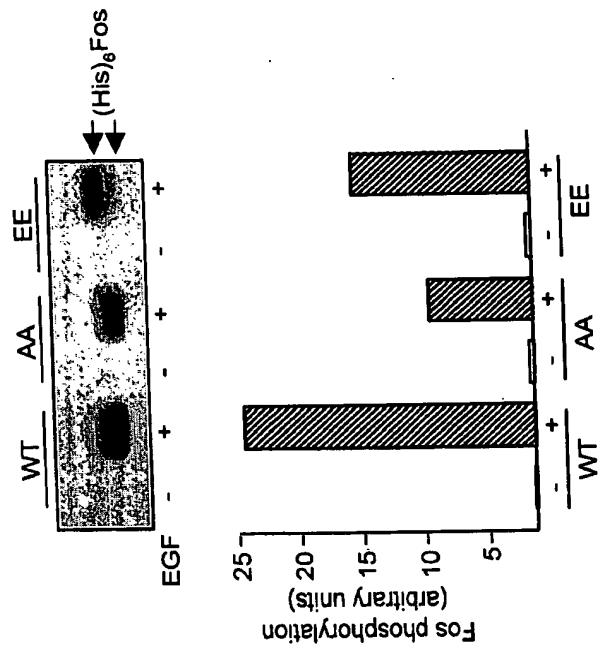


Figure 2

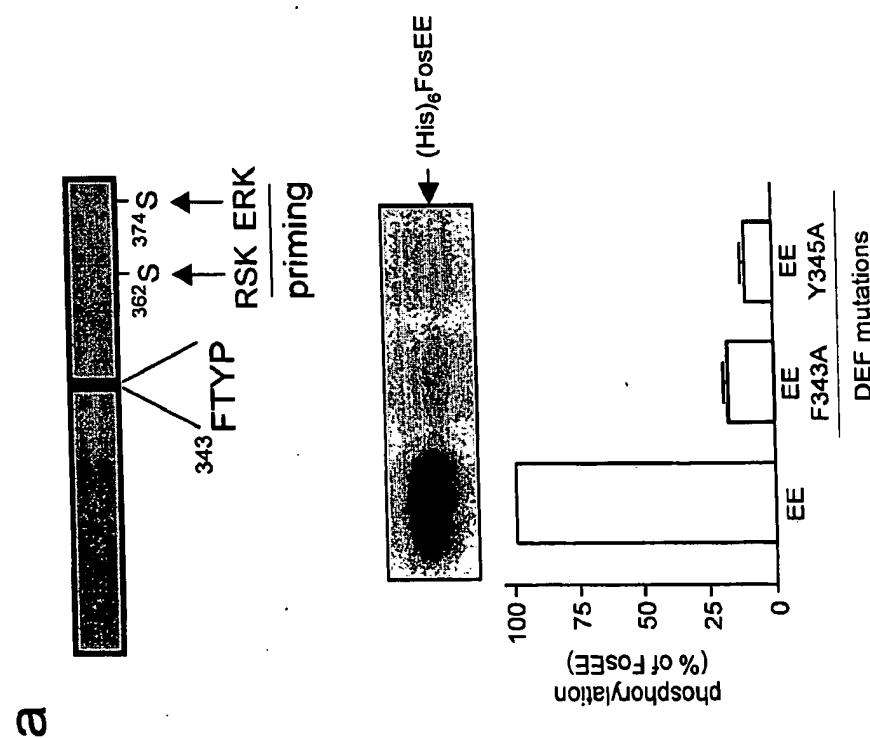
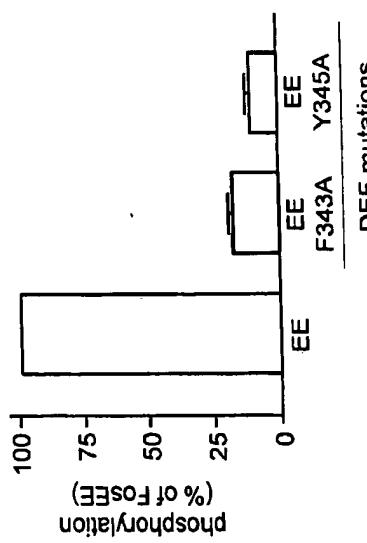


Figure 3



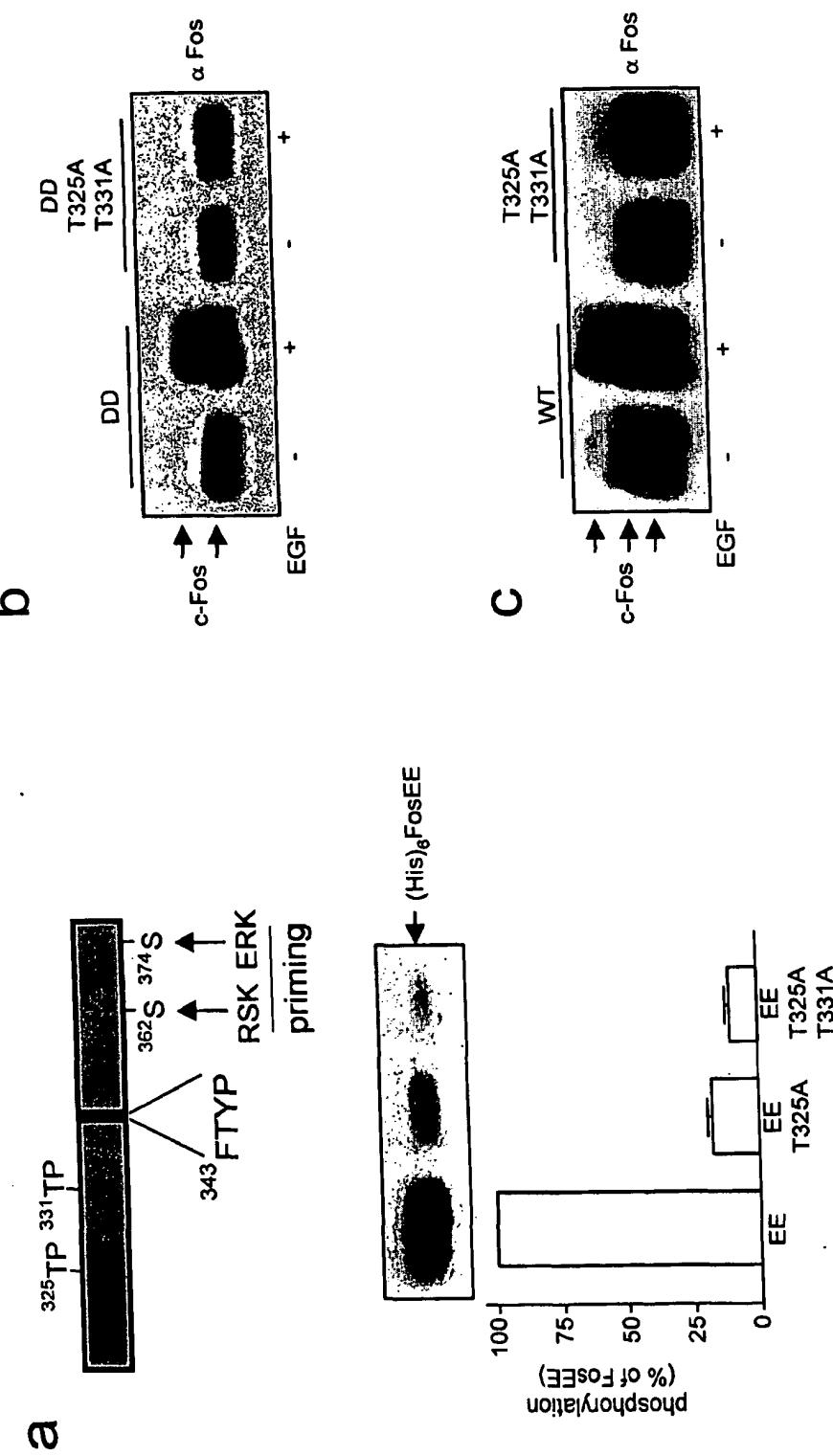
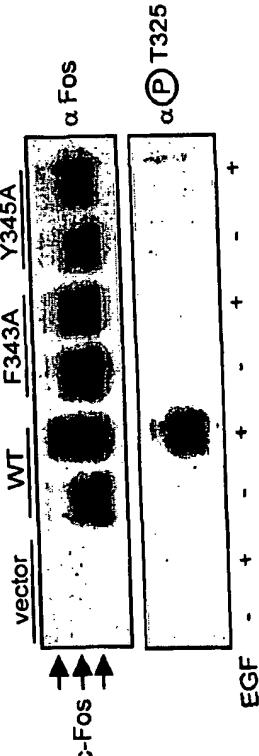


Figure 4

a PMVTELEPLCTPVWTCTPSCTTYY
①T325 epitope

d

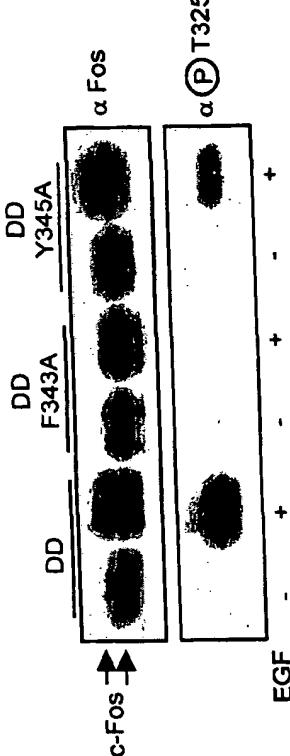


a

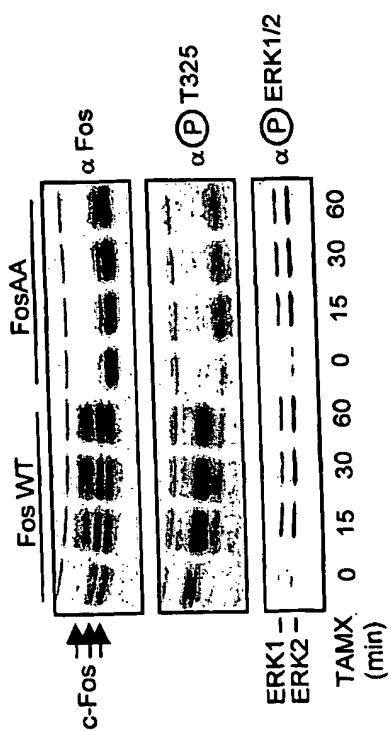
b



e

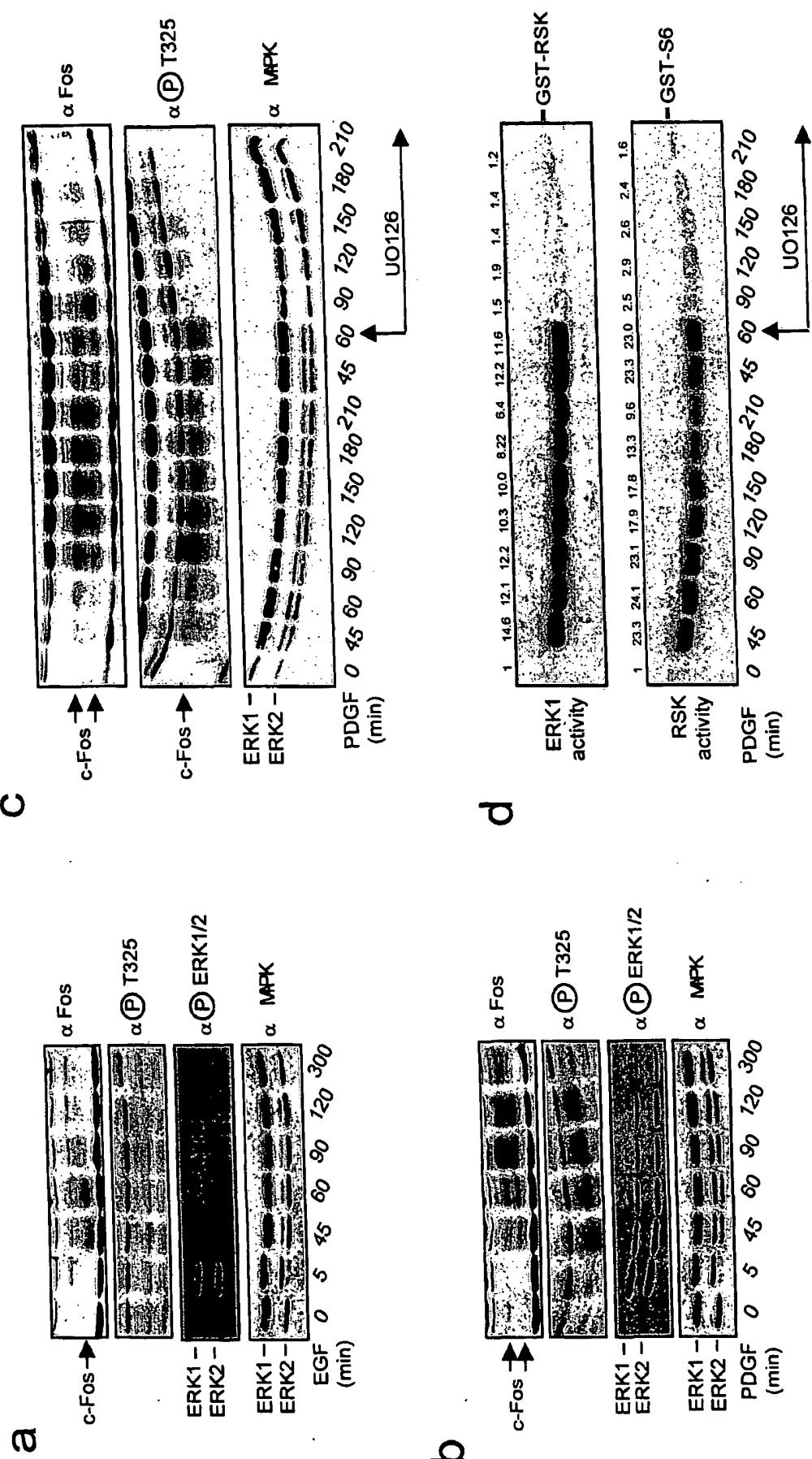


c



ERK1- =
ERK2- =
TAMX 0 15 30 60 0 15 30 60
(min) = = = = = = = =

Figure 5



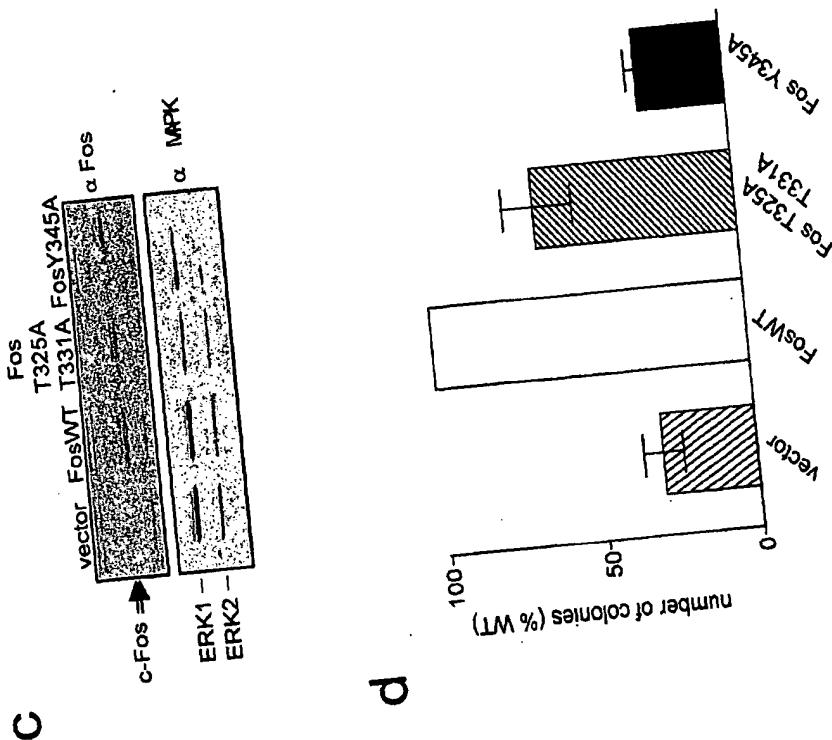
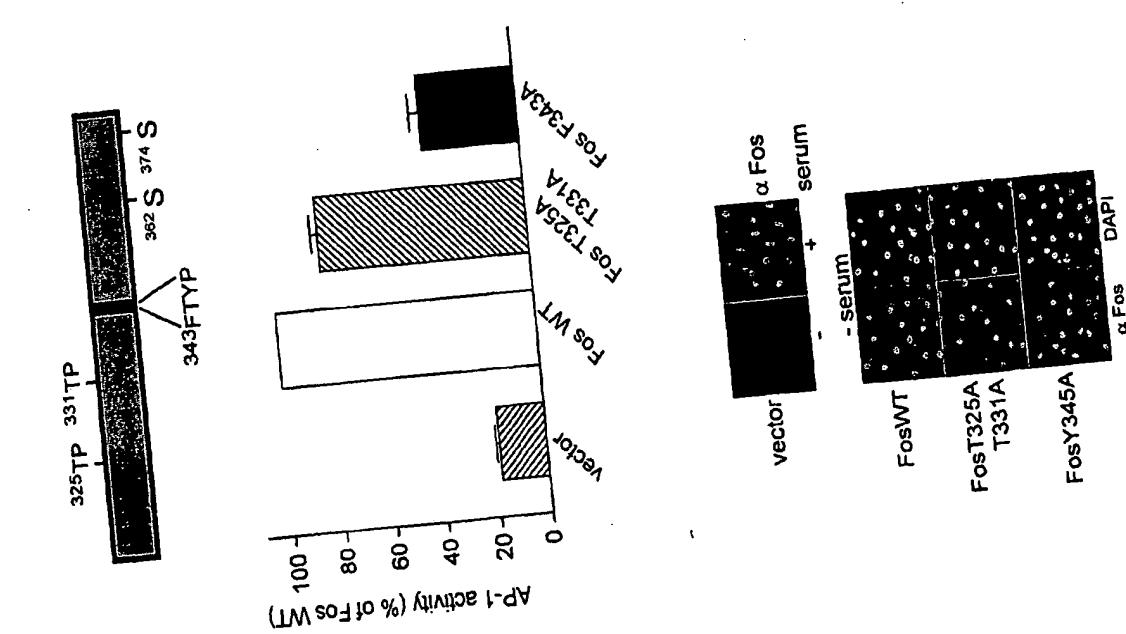
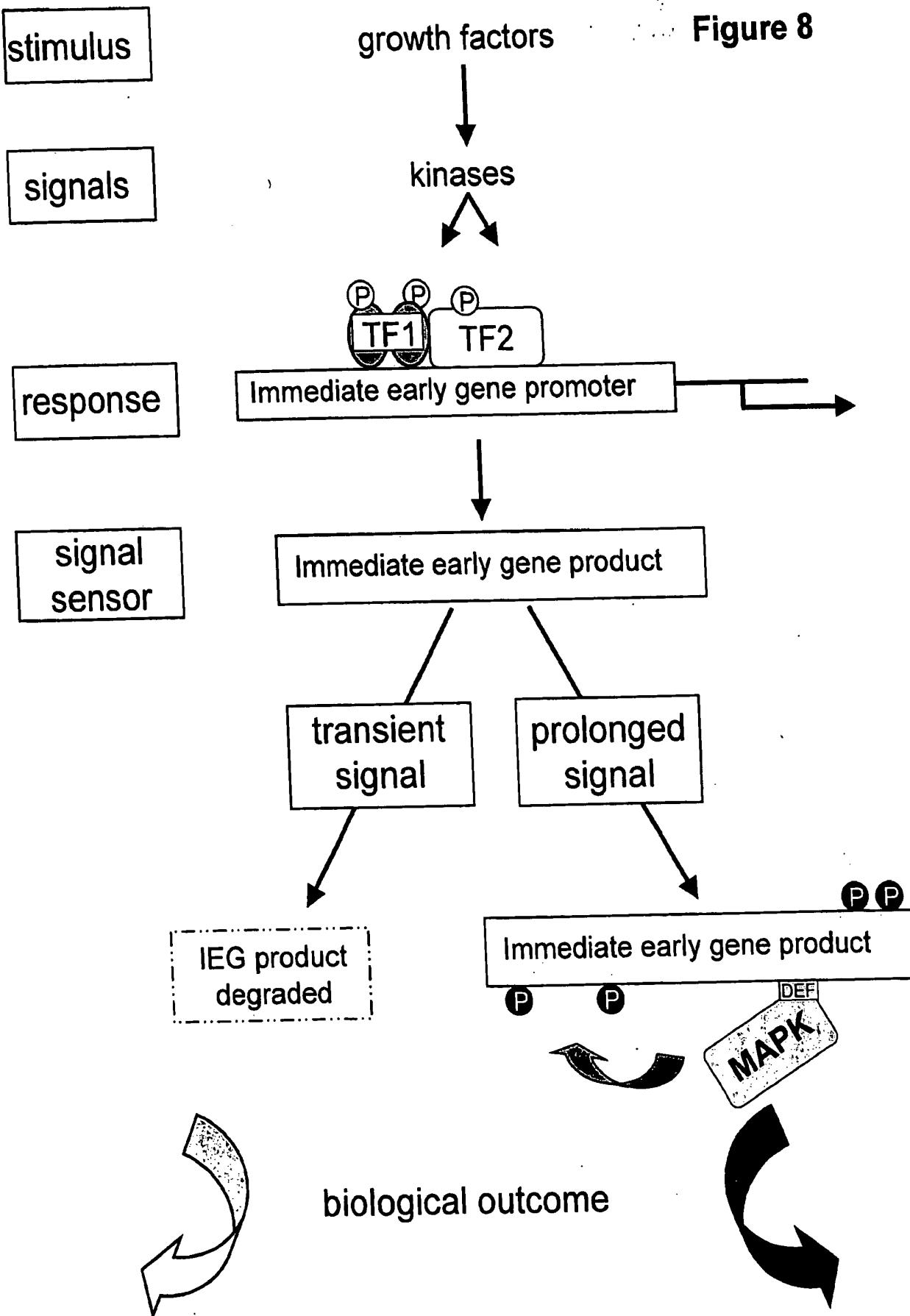


Figure 7

Figure 8



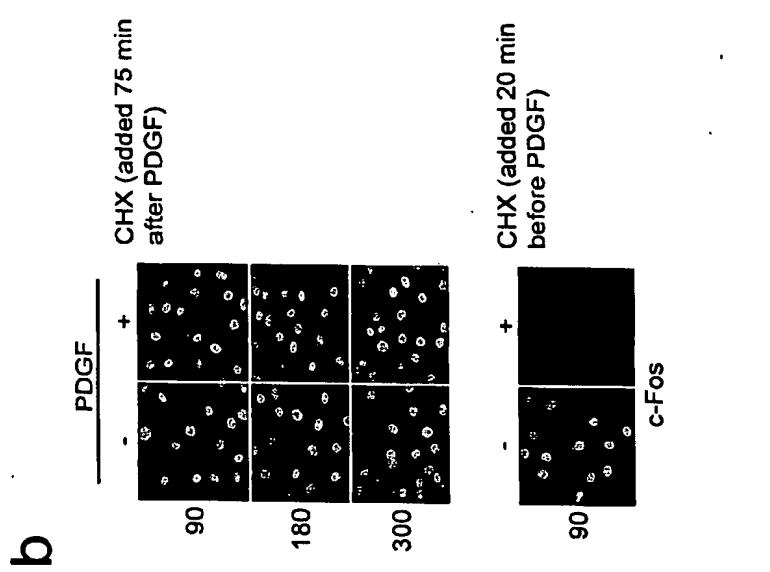
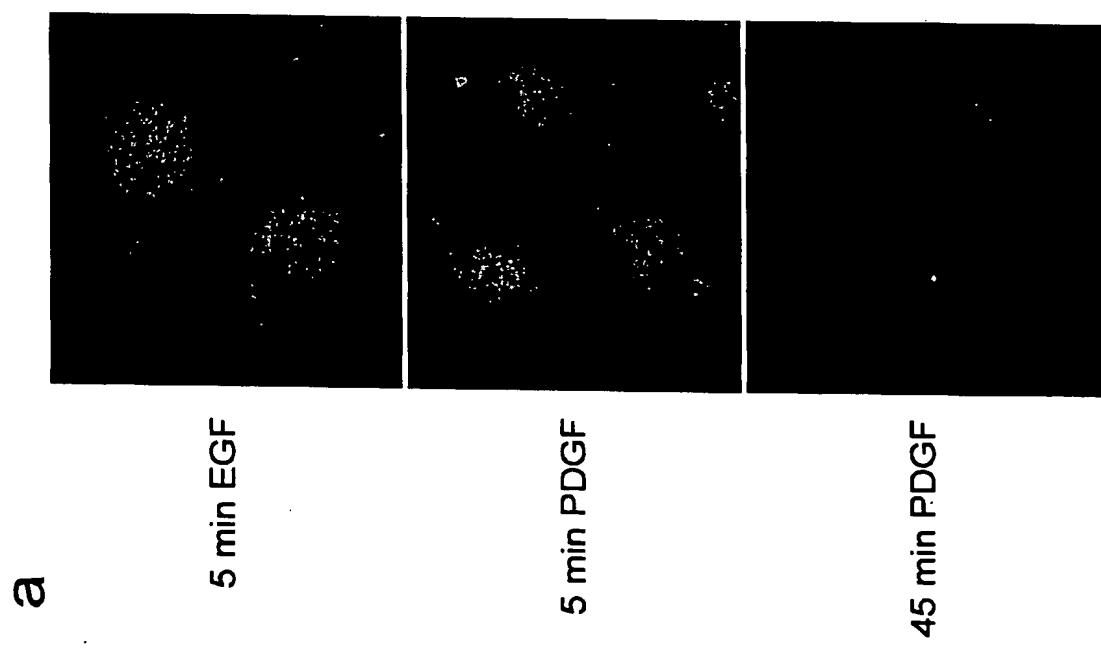
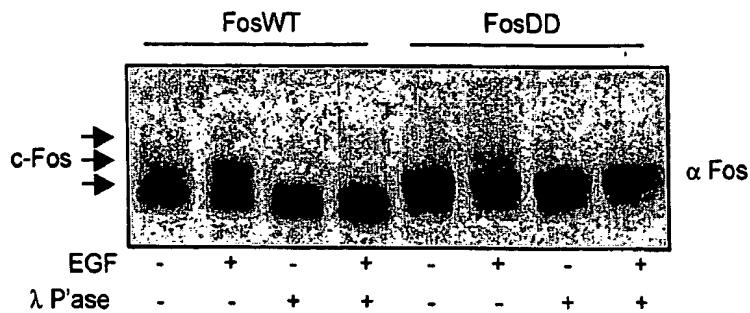


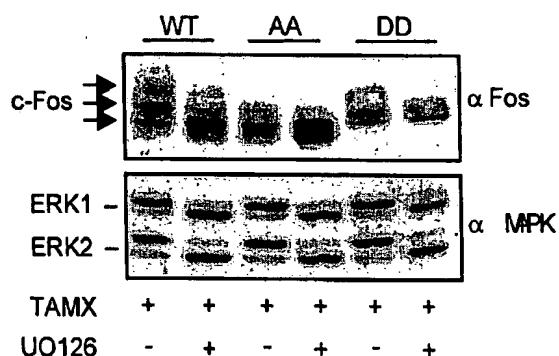
Figure 9

Figure 10

۲۰۰



b



C

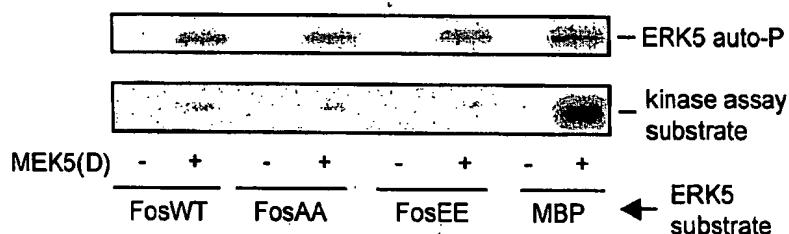


Figure 11

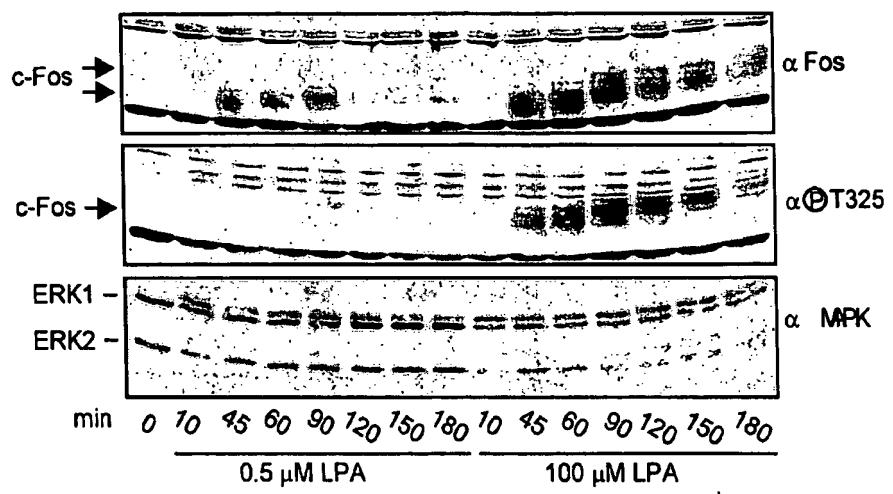


Figure 12

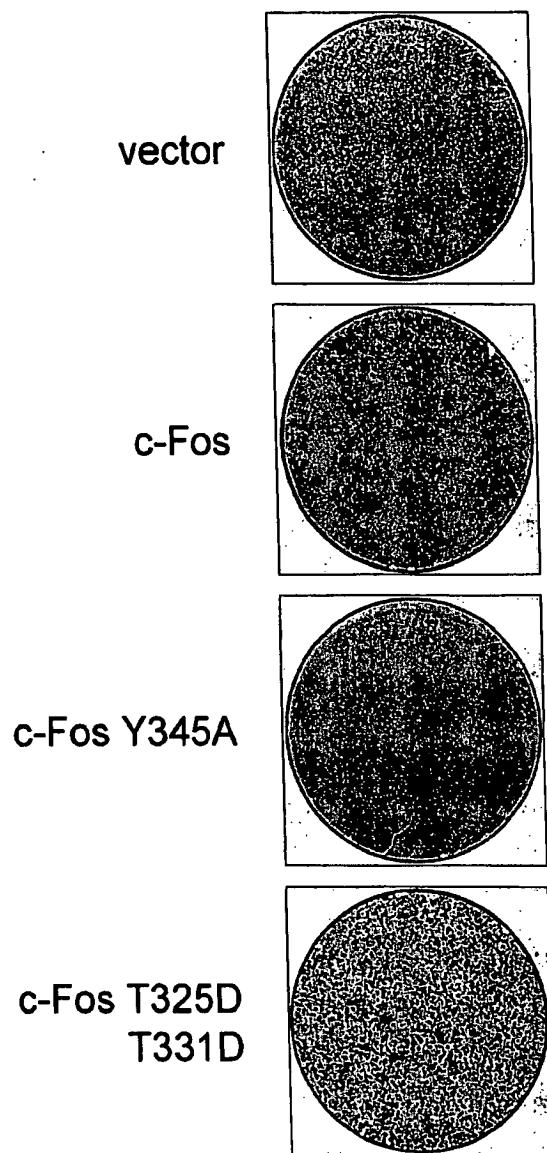
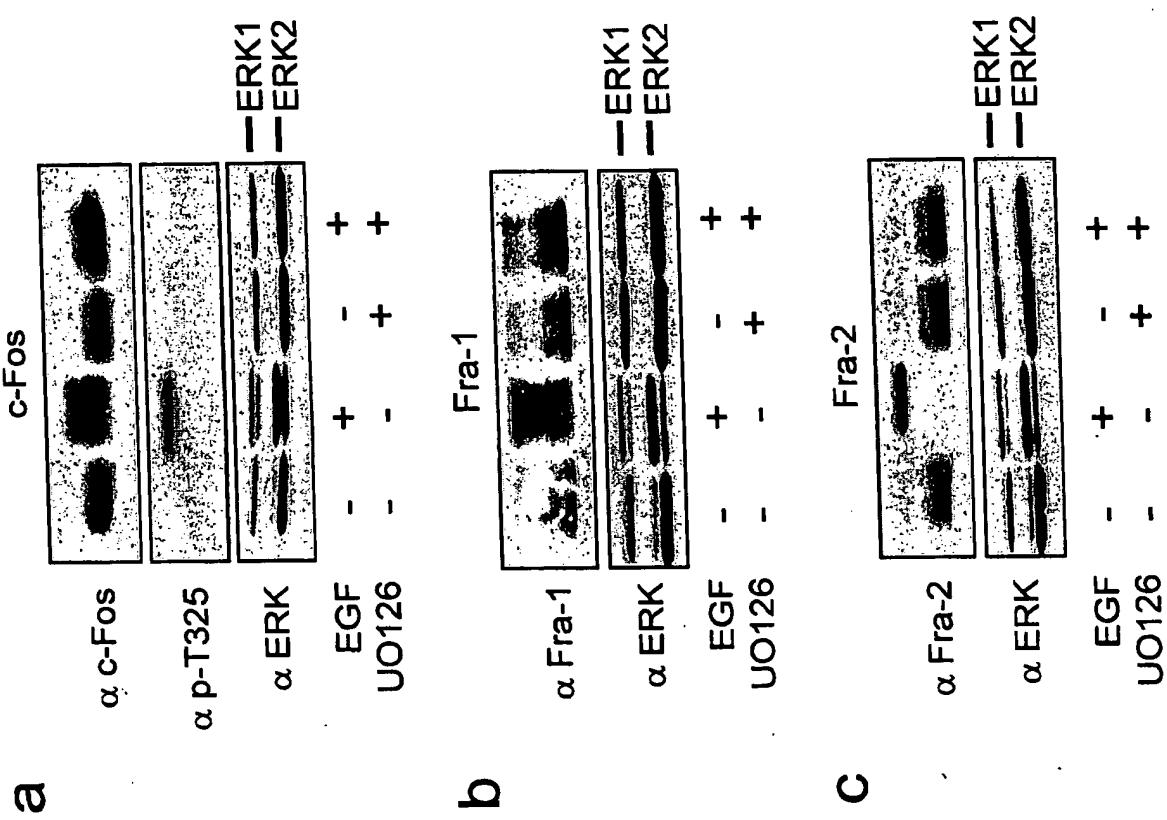


Figure 13



a

c-Fos GPMVTELEPLCTPVVTCITPSCTTYTSSSFVFTYP-----EADSFPSCAAHRKGSSS-NEPSSSDSISSSPTLLA
 Fra-2 ----GEEPLHTPIVVISTPAVTPGTSNLVFTYPSVLEQESPASPSESCSKAHRRSSSS-GDQSSSDSISNSPTLLA
 Fra-1 -GPVLEPEALHTPTLMTTPSLTPFTPSLVTYPS-----STIPEPCSSSTHRKSSSSSGDPSSDPLGSPTLLA

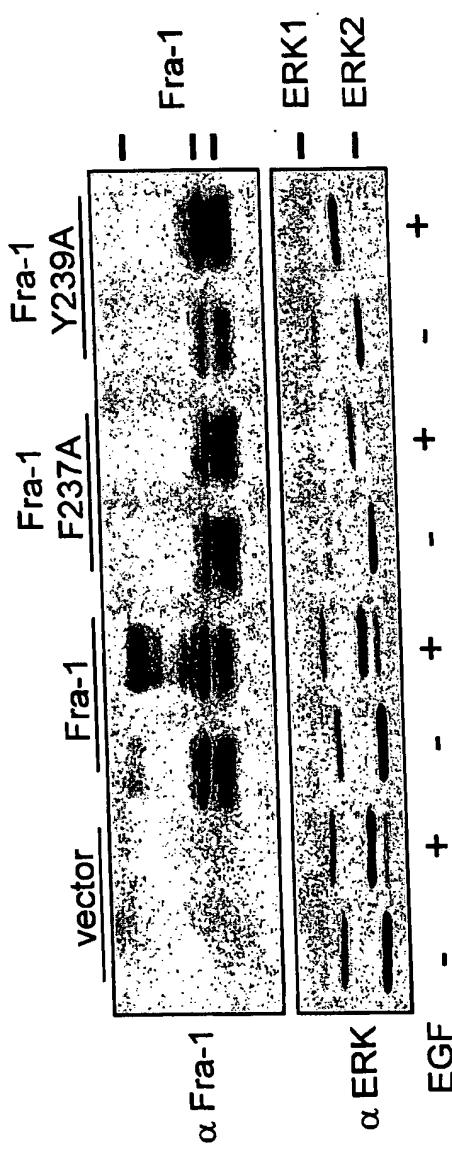
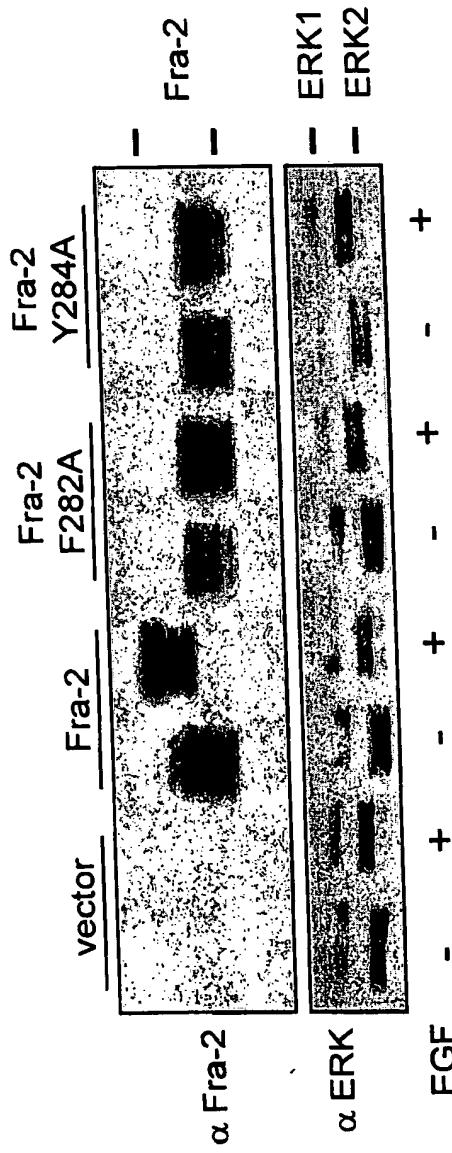
b**c**

FIGURE 15

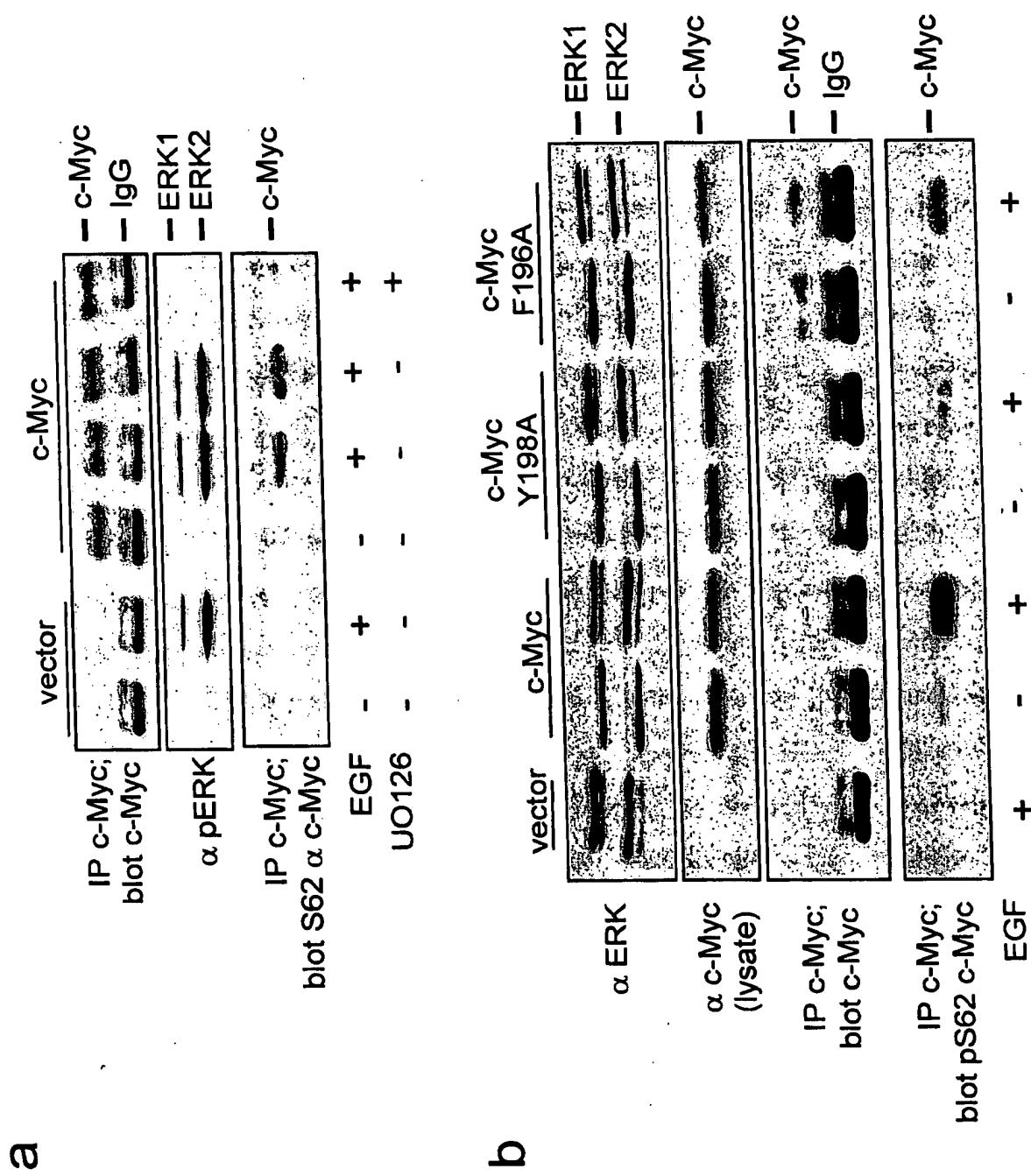


Figure 16

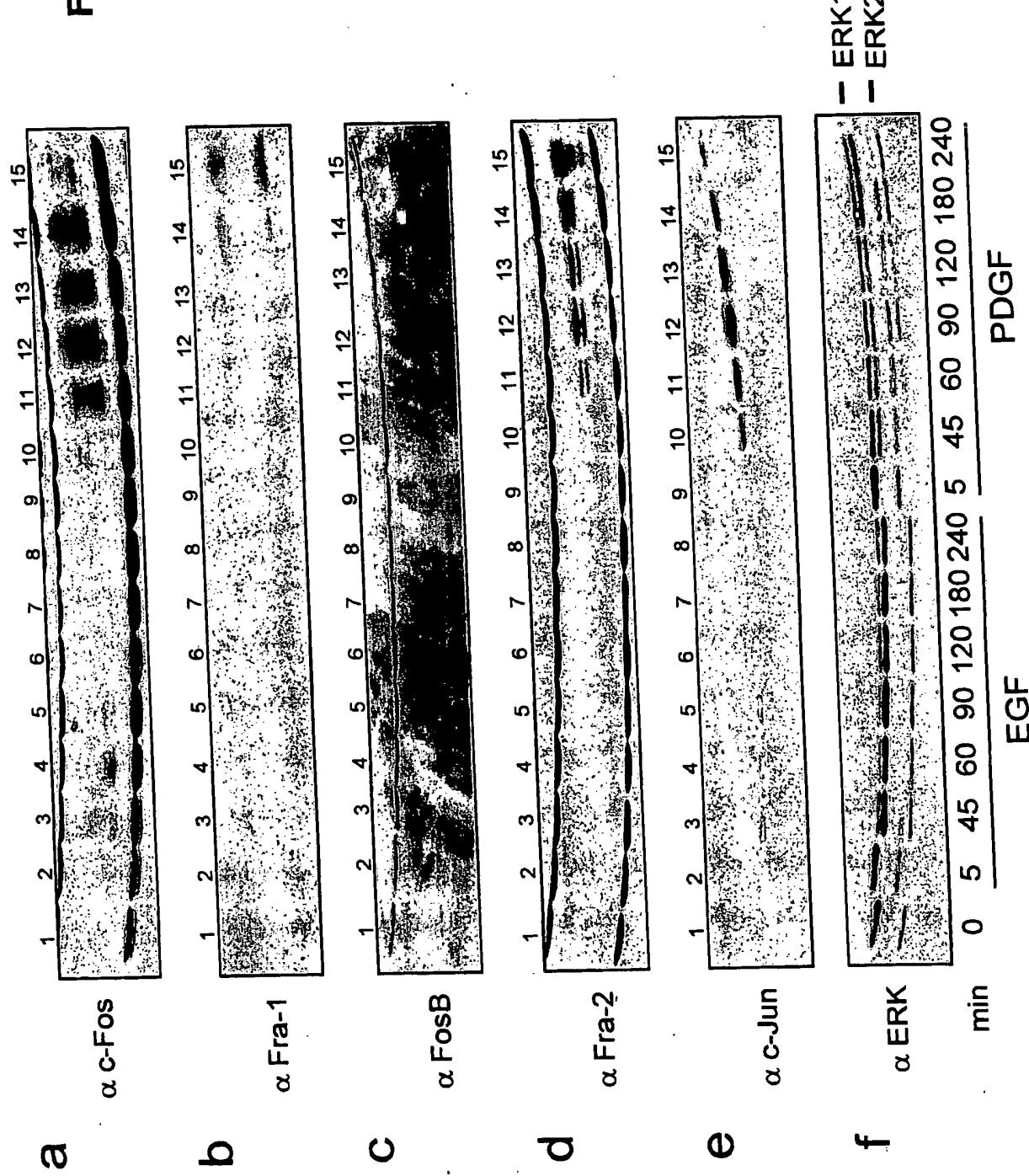


Figure 17

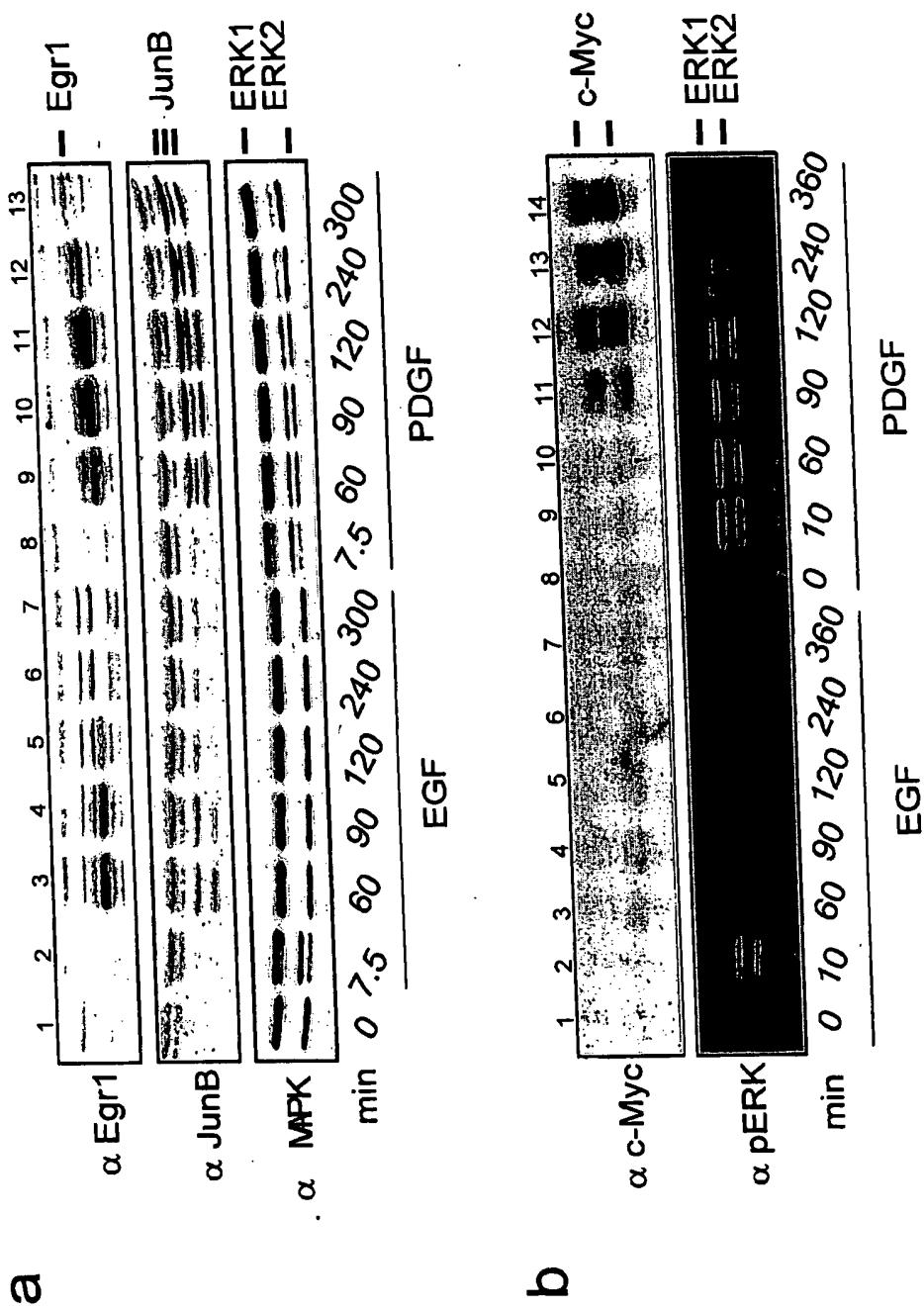
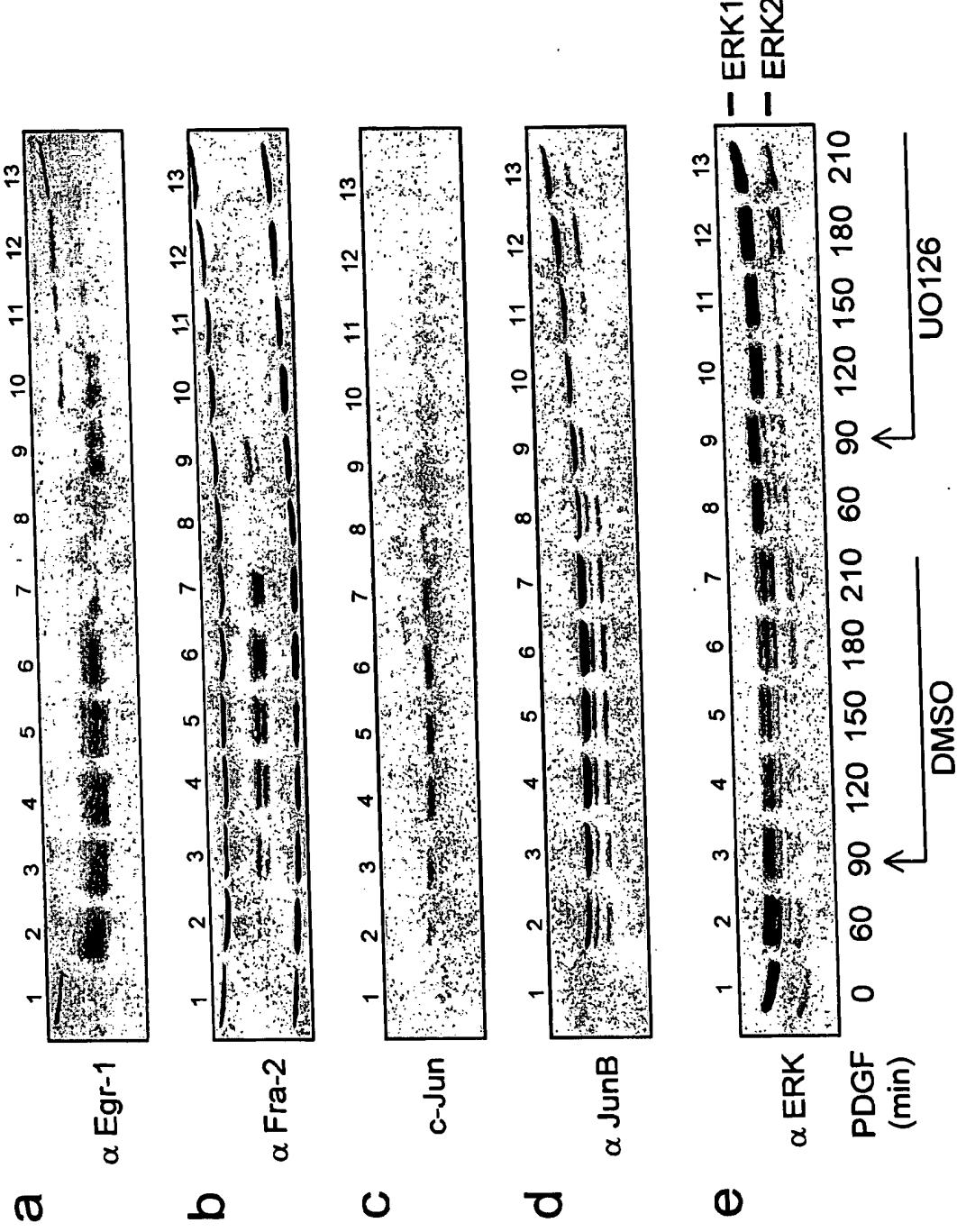


Figure 18



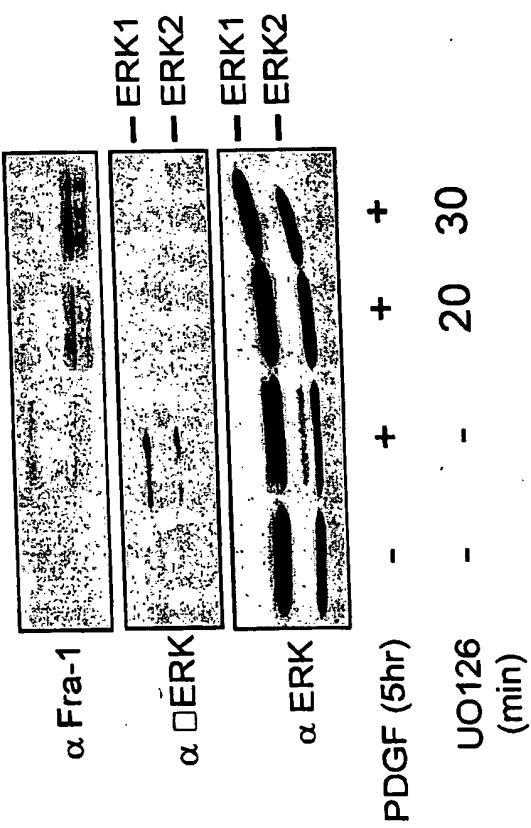
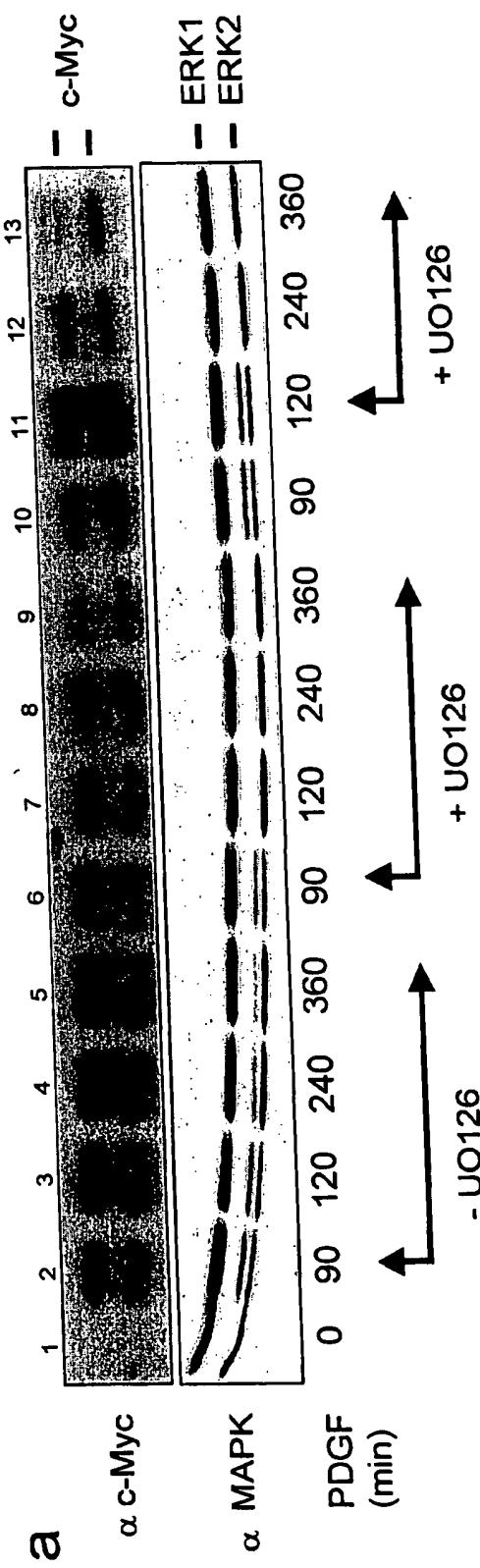


Figure 19

Figure 20

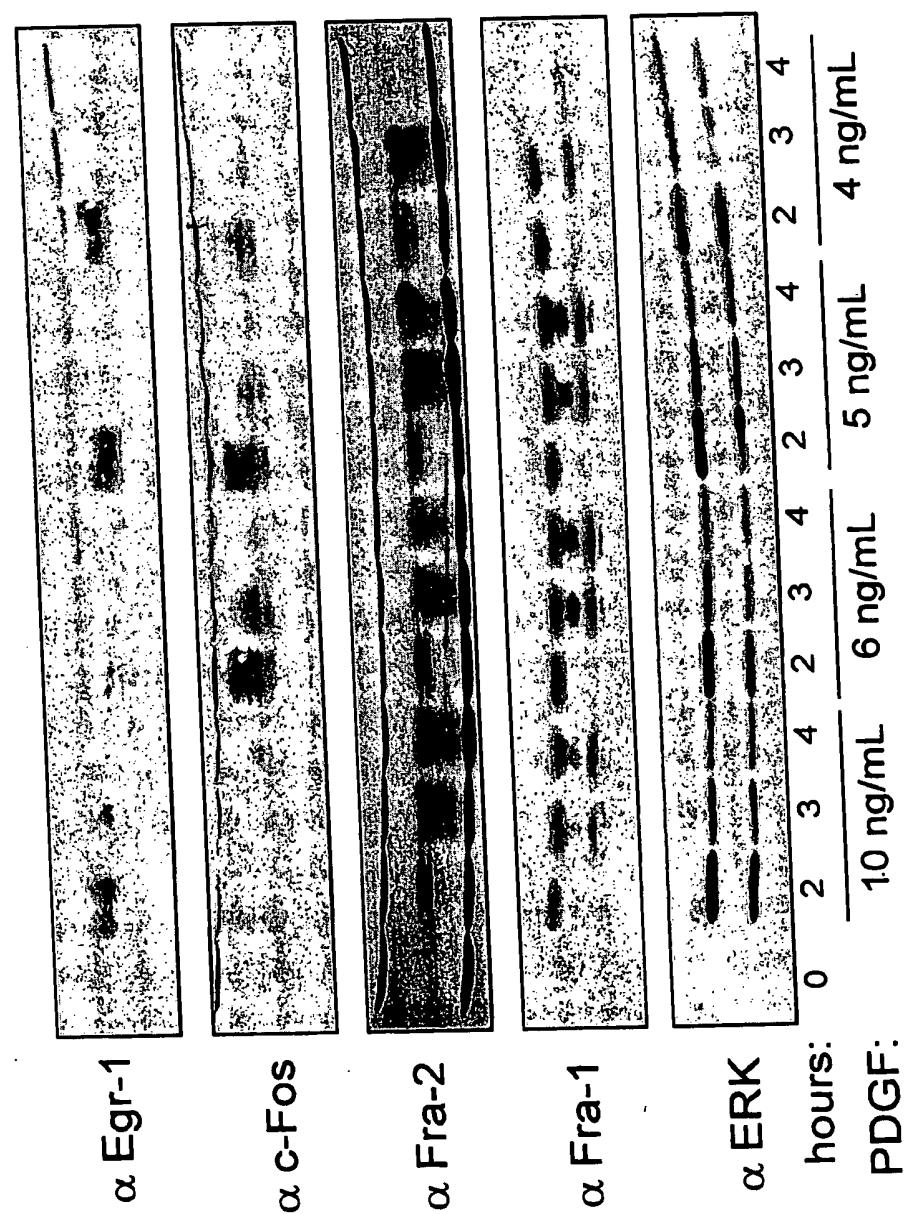


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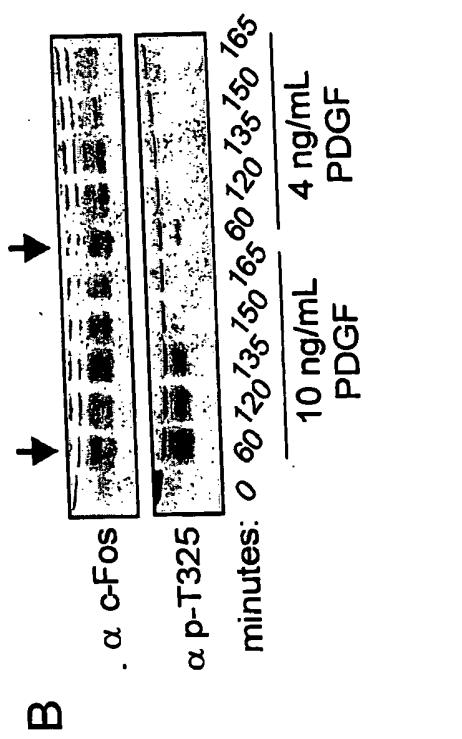
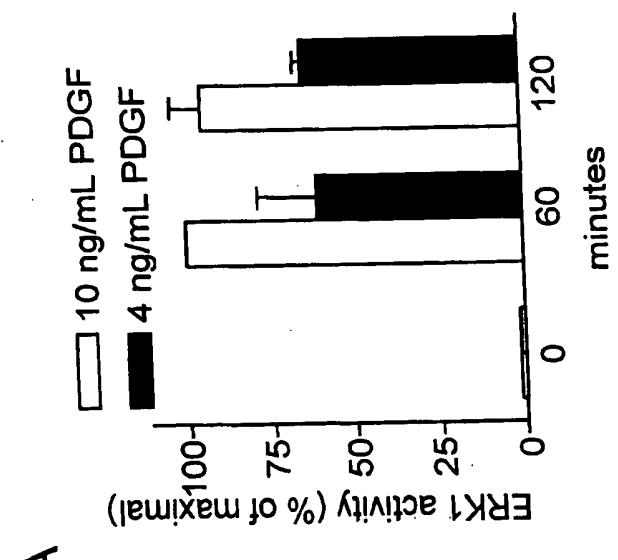


Figure 22

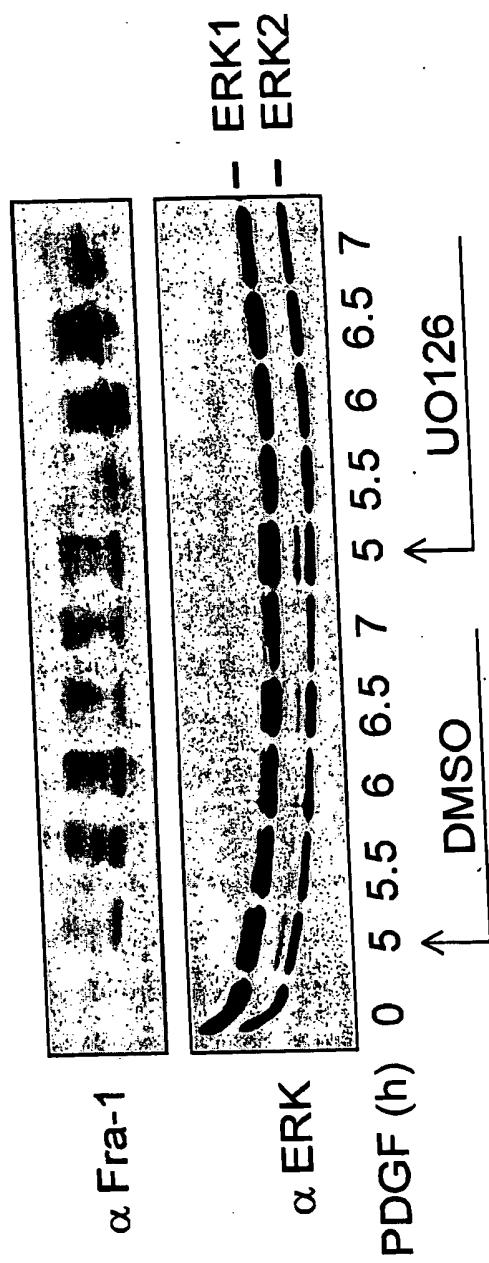


Figure 23

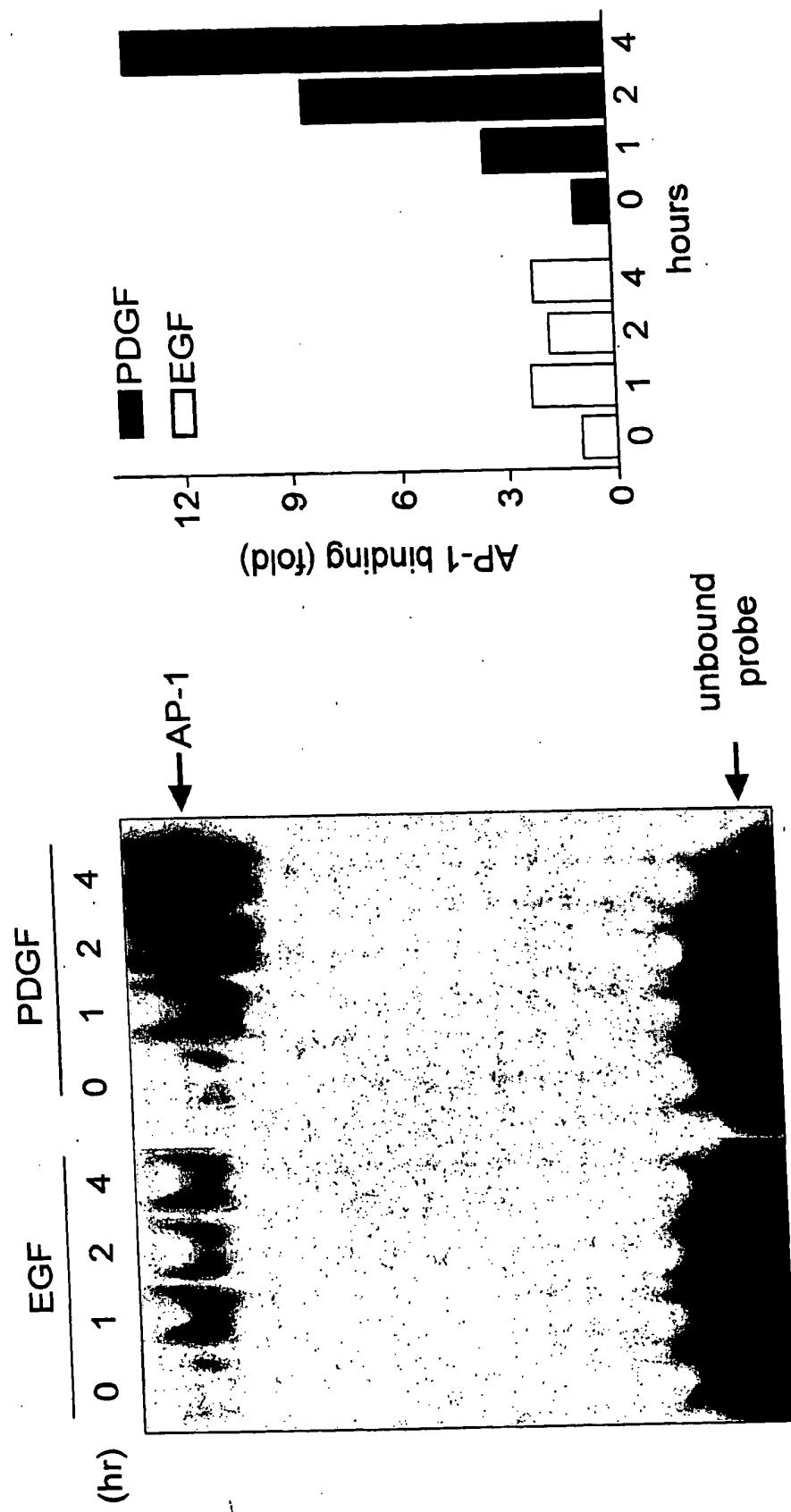
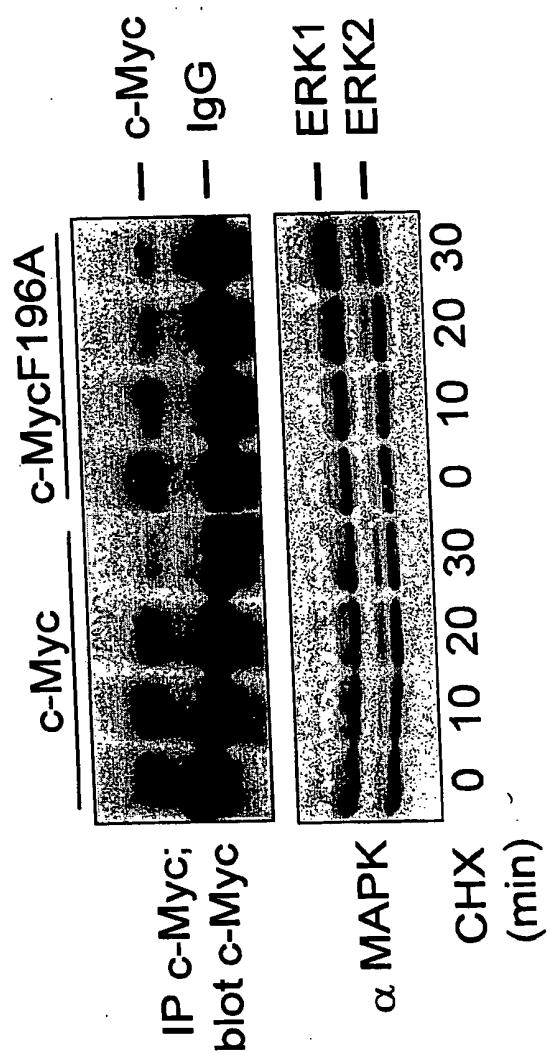


Figure 24



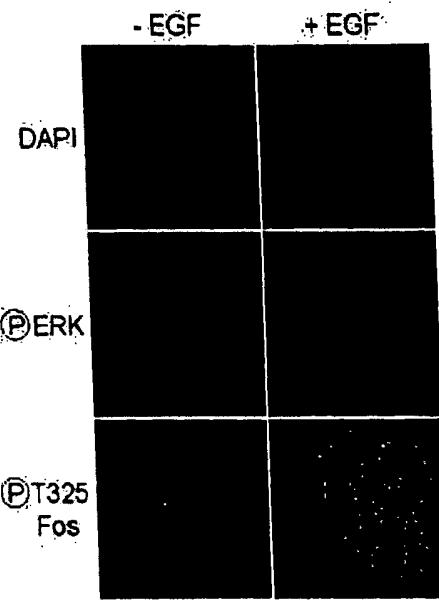


FIGURE 25

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